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Dated 15 July 2003

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23JUN02 E727504-1 C72481
P01/7700 0.00-0214268.5

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1. Your reference — PA 512

2. Patent application number *(The Patent Office will fill in this part)* — 0214268.5 — 20 JUN 2002

3. Full name, address and postcode of the or of each applicant *(underline all surnames)*

CELLTECH R&D LIMITED
208 BATH ROAD
SLOUGH
SL1 3WE

Patents ADP number *(if you know it)* 8104850001

If the applicant is a corporate body, give the country/state of its incorporation — UK

4. Title of the invention

CHEMICAL COMPOUNDS

5. Name of your agent *(if you have one)* —

"Address for service" in the United Kingdom to which all correspondence should be sent *(including the postcode)*

CELLTECH R&D LIMITED
PATENTS DEPARTMENT
208 BATH ROAD
SLOUGH
SL1 3WE

Patents ADP number *(if you know it)* 8182305001

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Country	Priority application number *(if you know it)*	Date of filing *(day / month / year)*
—	—	—

7. If this application is divided or otherwise derived from an earlier UK application, give the number and the filing date of the earlier application

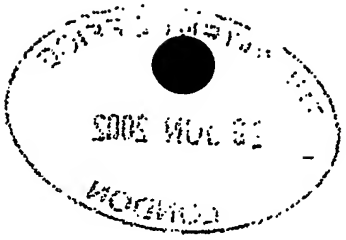
Number of earlier application	Date of filing *(day / month / year)*
—	—

8. Is a statement of inventorship and of right to grant of a patent required in support of this request? *(Answer 'Yes' if:*

a) any applicant named in part 3 is not an inventor, or
b) there is an inventor who is not named as an applicant, or
c) any named applicant is a corporate body.
See note (d))

YES

Patents Form 1/77



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Claim(s)	0
Abstract	0
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Priority documents	0
Translations of priority documents	0
Statement of inventorship and right to grant of a patent *(Patents Form 7/77)*	0
Request for preliminary examination and search *(Patents Form 9/77)*	0
Request for substantive examination *(Patents Form 10/77)*	0
Any other documents *(please specify)*	0

11. I/We request the grant of a patent on the basis of this application.

FOR & ON BEHALF OF CELLTECH R&D LIMITED

Signature Date

2016/02

12. Name and daytime telephone number of person to contact in the United Kingdom

DR P. ANSELL 01753 447566

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CHEMICAL COMPOUNDS

This invention relates to a series of 5-6 fused ring bicyclic heteroaromatic derivatives, to compositions containing them, to processes for their preparation and to their use in medicine.

Immune and inflammatory responses involve a variety of cell types with control and co-ordination of the various interactions occurring *via* both cell-cell contacts (e.g integrin interactions with their receptors) and by way of intercellular signalling molecules. A large number of different signalling molecules are involved including cytokines, lymphocytes, chemokines and growth factors.

Cells respond to such intercellular signalling molecules by means of intracellular signalling mechanisms that include protein kinases, phosphatases and phospholipases. There are five classes of protein kinase of which the major ones are the tyrosine kinases and the serine/threonine kinases [Hunter, T., Methods in Enzymology (Protein Kinase Classification) p. 3, Hunter, T. and Sefton, B.M.; eds. Vol. 200, Academic Press; San Diego, 1991].

One sub-class of serine/threonine kinases is the mitogen activating protein (MAP) kinases of which there are at least three families which differ in the sequence and size of the activation loop [Adams, J. L. *et al*, Progress in Medicinal Chemistry p. 1-60, King, F. D. and Oxford, A. W.; eds. vol 38, Elsevier Science, 2001]: the extracellular regulated kinases (ERKs), the c-Jun NH_2 terminal kinases or stress activated kinases (JNKs or SAP kinases) and the p38 kinases which have a threonine-glycine-tyrosine (TGY) activation motif. Both the JNKs and p38 MAP kinases are primarily activated by stress stimuli including, but not limited to, proinflammatory cytokines e.g. tumour necrosis factor (TNF) and interleukin-1 (IL-1), ultraviolet light, endotoxin and chemical or osmotic shock.

Four isoforms of p38 have been described (p38α/β/γ/δ). The human p38α enzyme was initially identified as a target of cytokine-suppressive anti-inflammatory drugs (CSAIDs) and the two isoenzymes found were initially termed CSAID binding protein-1 (CSBP-1) and CSBP-2 [Lee, J. C. *et al*, Nature (London) 1994; 372, 739-46]. CSBP-2 is now widely referred to as p38α and differs from CSBP-1 in an internal sequence of 25 amino acids as a result of differential splicing of two exons that are conserved in both mouse and human [McDonnell, P. C. et al, Genomics 1995, 29, 301-2]. CSBP-1 and p38α are expressed ubiquitously and there is no difference between the two isoforms with respect to tissue distribution, activation profile, substrate preference or CSAID binding. A second isoform is p38β which has 70% identity with p38α. A second form of p38β termed p38β2 is also known and of the two this is believed to be the major form. p38α and p38β2 are expressed in many different tissues. However in monocytes and macrophages p38α is the predominant kinase activity [Lee, J. C., *ibid*; Jing, Y. *et al*, J. Biol. Chem. 1996, 271, 10531-34; Hale, K. K. *et al*, J. Immun. 1999, 162, 4246-52]. p38γ and p38δ (also termed SAP kinase-3 and SAP kinase-4 respectively) have ~63% and ~61% homology to p38α respectively. p38γ is predominantly expressed in skeletal muscle whilst p38δ is found in testes, pancreas, prostate, small intestine and in certain endocrine tissues.

All p38 homologues and splice variants contain a 12 amino acid activation loop that includes a Thr-Gly-Tyr motif. Dual phosphorylation of both Thr-180 and Tyr-182 in the TGY motif by a dual specificity upstream kinase is essential for the activation of p38 and results in a >1000-fold increase in specific activity of these enzymes [Doza, Y. N. *et al* FEBS Lett., 1995, 364, 7095-8012]. This dual phosphorylation is effected by MKK6 and under certain conditions the related enzyme MKK3 [Enslen, H. *et al* J. Biol. Chem., 1998, 273, 1741-48]. MKK3 and MKK6 belong to a family of enzymes termed

MAPKK (mitogen activating protein kinase kinase) which are in turn activated by MAPKKK (mitogen activating kinase kinase kinase) otherwise known as MAP3K.

Several MAP3Ks have been identified that are activated by a wide variety of stimuli including environmental stress, inflammatory cytokines and other factors. MEKK4/MTK1 (MAP or ERK kinase kinase/MAP three kinase-1), ASK1 (apoptosis stimulated kinase) and TAK1 (TGF-β-activated kinase) are some of the enzymes identified as upstream activators of for MAPKKs. MEKK4/MTK1 is thought to be activated by several GADD-45-like genes that are induced in response to environmental stimuli and which eventually lead to p38 activation [Takekawa, M. and Saito, H. Cell, 1998, 95, 521-30]. TAK1 has been shown to activate MKK6 in response to transforming growth factor-β (TGF-β). TNF-stimulated activation of p38 is believed to be mediated by the recruitment of TRAF2 [TNF receptor associated factor] and the Fas adaptor protein, Daxx, which results in the activation of ASK1 and subsequently p38.

Several substrates of p38 have been identified including other kinases [e.g. MAPK activated protein kinase 2/3/5 (MAPKAP 2/3/5), p38 regulated/activated protein kinase (PRAK), MAP kinase-interacting kinase 1/2 (MNK1/2), mitogen- and stress-activated protein kinase 1 (MSK1/RLPK) and ribosomal S6 kinase-B (RSK-B)], transcription factors [e.g. activating transcription factor 2/6 (ATF2/6), monocyte-enhancer factor-2A/C (MEF2A/C), C/EBP homologous protein (CHOP), Elk1 and Sap-1a1] and others substrates [e.g. cPLA2, p47phox].

MAPKAP K2 is activated by p38 in response to environmental stress. Mice engineered to lack MAPKAP K2 do not produce TNF in response to lipopolysaccharide (LPS). Production of several other cytokines such as IL-1, IL-6, IFN-g and IL-10 is also partially inhibited [Kotlyarov, A. *et al* Nature Cell Biol. 1999, 1, 94-7]. Further, MAPKAP K2 from embryonic stem cells from

p38α null mice was not activated in response to stress and these cells did not produce IL-6 in response to IL-1 [Allen, M. *et al*, J. Exp. Med. 2000, 191, 859-69]. These results indicate that MAPKAP K2 is not only essential for TNF and IL-1 production but also for signalling induced by cytokines. In addition MAPKAP K2/3 phosphorylate and thus regulate heat shock proteins HSP 25 and HSP 27 which are involved in cytoskeletal reorganization.

Several small molecule inhibitors of p38 have been reported which inhibit IL-1 and TNF synthesis in human monocytes at concentrations in the low μM range [Lee, J. C. *et al*, Int. J. Immunopharm. 1988, 10, 835] and exhibits activity in animal models which are refactory to cyclooxygenase inhibitors [Lee, J. C. et al, Annals N. Y. Acad. Sci. 1993, 696, 149]. In addition these small molecule inhibitors are known to also decrease the synthesis of a wide variety of pro-inflammatory proteins including IL-6, IL-8, granulocyte/macrophage colony-stimulating factor (GM-CSF) and cyclooxygenase-2 (COX-2). TNF-induced phosphorylation and activation of cytosolic PLA2, TNF-induced expression of VCAM-1 on endothelial cells and IL-1 stimulated synthesis of collagenase and stromelysin are also inhibited by such small molecule inhibitors of p38 [Cohen, P. Trends Cell Biol. 1997, 7, 353-61].

A variety of cells including monocytes and macrophages produce TNF and IL-1. Excessive or unregulated TNF production is implicated in a number of disease states including Crohn's disease, ulcerative colitis, pyresis, rheumatoid arthritis, rheumatoid spondylitis, osteoarthritis, gouty arthritis and other arthritic conditions, toxic shock syndrome, endotoxic shock, sepsis, septic shock, gram negative sepsis, bone resporption diseases, reperfusion injury, graft vs. host reaction, allograft rejection, adult respiratory distress syndrome, chronic pulmonary inflammatory disease, silicosis, pulmonary sarcoisosis, cerebral malaria, scar tissue formation, keloid formation, fever and myalgias due to infection, such as influenza, cachexia secondary to

acquired immune deficiency syndrome (AIDS), cachexia secondary to infection or malignancy, AIDS or AIDS related complex.

Excessive or unregulated IL-1 production has been implicated in rheumatoid arthritis, osteoarthritis, traumatic arthritis, rubella arthritis, acute synovitis, psoriatic arthritis, cachexia, Reiter's syndrome, endotoxemia, toxic shock syndrome, tuberculosis, atherosclerosis, muscle degeneration, and other acute or chronic inflammatory diseases such as the inflammatory reaction induced by endotoxin or inflammatory bowel disease. In addition IL-1 has been linked to diabetes and pancreatic β cells [Dinarello, C. A. J. Clinical Immunology, 1985, 5, 287-97].

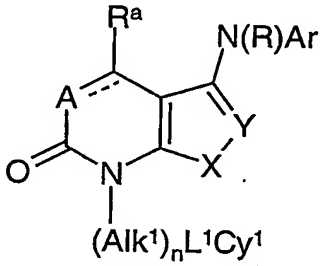
IL-8 is a chemotactic factor produced by various cell types including endothelial cells, mononuclear cells, fibroblasts and keratinocytes. IL-1, TNF and LPS all induce the production of IL-8 by endothelial cells. *In vitro* IL-8 has been shown to have a number of functions including being a chemoattractant for neutrophils, T-lymphocytes and basophils. IL-8 has also been shown to increase the surface expression of Mac-1 (CD11b/CD18) on neutrophils without *de novo* protein synthesis which may contribute to increased adhesion of neutrophils to vascular endothelial cells. Many diseases are characterised by massive neutrophil infiltration. Histamine release from basophils (in both atopic and normal individuals) is induced by IL-8 as is lysozomal enzyme release and respiratory burst from neutrophils.

The central role of IL-1 and TNF together with other leukocyte derived cytokines as important and critical inflammatory mediators is well documented. The inhibition of these cytokines has been shown or would be expected to be of benefit in controlling, alleviating or reducing many of these disease states.

The central position that p38 occupies within the cascade of signalling molecules mediating extracellular to intracellular signalling (see Figure 1) and its influence over not only IL-1, TNF and IL-8 production but also the synthesis and/or action of other pro-inflammatory proteins (e.g. IL-6, GM-CSF, COX-2, collagenase and stromelysin) make it an attractive target for inhibition by small molecule inhibitors with the expectation that such inhibition would be a highly effective mechanism for regulating the excessive and destructive activation of the immune system. Such an expectation is supported by the potent and diverse anti-inflammatory activities described for p38 kinase inhibitors [Adams, *ibid*; Badger, *et al*, J. Pharm. Exp. Ther. 1996, 279, 1453-61; Griswold, *et al*, Pharmacol. Comm., 1996, 7, 323-29].

We have now found a group of compounds which are potent and selective inhibitors of p38 kinase (p38α, β, δ and γ) and the isoforms and splice variants thereof, especially p38α, p38β and p38β2. The compounds are thus of use in medicine, for example in the prophylaxis and treatment of immune or inflammatory disorders as described herein.

Thus according to one aspect of the invention we provide a compound of formula (1):



(1)

wherein:

the dashed line joining A and C(R^a) is present and represents a bond and

A is a –N= atom or a –C(R^b)= group, or the dashed line is absent and A is a -N(R^b)-, or –C(R^b)(R^c)- group;

R^a, R^b and R^c is each independently a hydrogen atom or an optionally substituted C_{1-6}alkyl, -CN, -CO_2H, -CO_2R^1 (where R^1 is an optionally substituted alkyl group), -$CONH_2$, -$CONHR^1$ or -$CONR^1R^2$ group (where R^2 is an optionally substituted alkyl group);

R is a hydrogen atom or a straight or branched C_{1-6} alkyl group;

X is an -O-, -S- or substituted nitrogen atom or a -S(O)-, -S(O_2)- or -NH- group;

Y is a nitrogen or substituted carbon atom or a –CH= group;

n is zero or the integer 1;

Alk^1 is an optionally substituted aliphatic or heteroaliphatic chain

L^1 is a covalent bond or a linker atom or group;

Cy^1 is a hydrogen atom or an optionally substituted cycloaliphatic, polycycloaliphatic, heterocycloaliphatic, polyheterocycloaliphatic, aromatic or heteroaromatic group;

Ar is an optionally substituted aromatic or heteroaromatic group;

and the salts, solvates, hydrates and N-oxides thereof;

It will be appreciated that compounds of formula (1) may have one or more chiral centres, and exist as enantiomers or diastereomers. The invention is to be understood to extend to all such enantiomers, diastereomers and mixtures thereof, including racemates. Formula (1) and the formulae hereinafter are intended to represent all individual isomers and mixtures thereof, unless stated or shown otherwise. In addition, compounds of formula (1) may exist as tautomers, for example keto (CH_2C=O)-enol (CH=CHOH) tautomers. Formula (1) and the formulae hereinafter are intended to represent all individual tautomers and mixtures thereof, unless stated otherwise.

As used in formula (1) the terms "substituted nitrogen atom" and "substituted carbon atom" are intended to include groups such as those in which X is

$-N(R^{10})-$ and Y is $-C(R^{10})=$ where R^{10} is a substituent other than a hydrogen atom as generally or particularly defined hereinafter.

The following general terms as used herein in relation to compounds of the invention and intermediates thereto have the stated meaning below unless specifically defined otherwise.

Thus as used herein the term "alkyl" whether present as a group or part of a group includes straight or branched C_{1-6}alkyl groups, for example C_{1-4}alkyl groups such as methyl, ethyl, n-propyl, i-propyl, n-butyl, s-butyl, i-butyl or t-butyl groups. Similarly, the terms "alkenyl" or "alkynyl" are intended to mean straight or branched C_{2-6}alkenyl or C_{2-6}alkynyl groups such as C_{2-4}alkenyl or C_{2-4}alkynyl groups. Optional substituents which may be present on these groups include those optional substituents mentioned hereinafter in relation to Alk^1 when Alk^1 is an optionally substituted aliphatic chain.

The term halogen is intended to include fluorine, chlorine, bromine or iodine atoms.

The term "haloalkyl" is intended to include those alkyl groups just mentioned sustituted by one, two or three of the halogen atoms just described. Particular examples of such groups include $-CF_3$, $-CCl_3$, $-CHF_2$, $-CHCl_2$, $-CH_2F$ and $-CH_2Cl$ groups.

The term "alkoxy" as used herein is intended to include straight or branched C_{1-6}alkoxy e.g. C_{1-4}alkoxy such as methoxy, ethoxy, n-propoxy, i-propoxy, n-butoxy, s-butoxy, i-butoxy and t-butoxy. "Haloalkoxy" as used herein includes any of these alkoxy groups substituted by one, two or three halogen atoms as described above. Particular examples include $-OCF_3$, $-OCCl_3$, $-OCHF_2$, $-OCHCl_2$, $-OCH_2F$ and $-OCH_2Cl$ groups.

As used herein the term "alkylthio" is intended to include straight or branched C_{1-6}alkylthio, e.g. C_{1-4}alkylthio such as methylthio or ethylthio.

As used herein the term "alkylamino or dialkylamino" is intended to include the groups $-NHR^{1a}$ and $-N(R^{1a})(R^{1b})$ where R^{1a} and R^{1b} is each independently an optionally substituted straight or branched alkyl group or both together with the N atom to which they are attached form an optionally substituted heterocycloalkyl group which may contain a further heteroatom or heteroatom containing group such as an –O- or -S- atom or $-N(R^{1a})$- group. Particular examples of such optionally substituted heterocycloalkyl groups include optionally substituted pyrrolidinyl, piperidinyl, morpholinyl, thiomorpholinyl and N'-C_{1-6}alkyl-piperazinyl groups. The optional substituents which may be present on such heterocycloalkyl groups include those optional substituents as described hereinafter in relation to aliphatic chains such as Alk^1.

When Alk^1 is present in compounds of formula (1) as an optionally substituted aliphatic chain it may be an optionally substituted C_{1-10}aliphatic chain. Particular examples include optionally substituted straight or branched chain C_{1-6}alkylene, C_{2-6}alkenylene, or C_{2-6}alkynylene chains.

Particular examples of aliphatic chains represented by Alk^1 include optionally substituted $-CH_2-$, $-CH_2CH_2-$, $-CH(CH_3)CH_2-$, $-(CH_2)_2CH_2-$, $-(CH_2)_3CH_2-$, $-CH(CH_3)(CH_2)_2CH_2-$, $-CH_2CH(CH_3)CH_2-$, $-C(CH_3)_2CH_2-$, $-CH_2C(CH_3)_2CH_2-$, $-(CH_2)_2CH(CH_3)CH_2-$, $-CH(CH_3)CH_2CH_2-$, $-CH(CH_3)CH_2CH(CH_3)CH_2-$, $-CH_2CH(CH_3)CH_2CH_2-$, $-(CH_2)_2C(CH_3)_2CH_2-$, $-(CH_2)_4CH_2-$, $-(CH_2)_5CH_2-$, $-CHCH-$, $-CHCHCH_2-$, $-CH_2CHCH-$, $-CHCHCH_2CH_2-$, $-CH_2CHCHCH_2-$, $-(CH_2)_2CHCH-$, $-CC-$, $-CCCH_2-$, $-CH_2CC-$, $-CCCH_2CH_2-$, $-CH_2CCCH_2-$ or $-(CH_2)_2CCH-$ chains.

Heteroaliphatic chains represented by Alk^1 in the compounds of formula (1) include the aliphatic chains just described but with each additionally containing one, two, three or four heteroatoms or heteroatom-containing groups. Particular heteroatoms or groups include atoms or groups L^2 where L^2 is a linker atom or group. Each L^2 atom or group may interrupt the aliphatic group, or may be positioned at its terminal carbon atom to connect the group to an adjoining atom or group. Particular examples include optionally substituted $-L^2CH_2-$, $-CH_2L^2-$, $-L^2CH(CH_3)-$, $-CH(CH_3)L^2-$, $-CH_2L^2CH_2-$, $-L^2CH_2CH_2-$, $-L^2CH_2CH(CH_3)-$, $-CH(CH_3)CH_2L^2-$, $-CH_2CH_2L^2-$, $-CH_2L^2CH_2CH_2-$, $-CH_2L^2CH_2CH_2L^2-$, $-(CH_2)_2L^2CH_2-$, $-(CH_2)_3L^2CH_2-$, $-L^2(CH_2)_2CH_2-$, $-L^2CH_2CHCH-$, $-CHCHCH_2L^2-$ and $-(CH_2)_2L^2CH_2CH_2-$ chains.

When L^2 is present in heteroaliphatic chains as a linker atom or group it may be any divalent linking atom or group. Particular examples include –O- or -S- atoms or –C(O)-, -C(O)O-, -OC(O)-, -C(S)-, -S(O)-, $-S(O)_2-$, $-N(R^3)-$ [where R^3 is a hydrogen atom or a straight or branched alkyl group], $-N(R^3)O-$, $-N(R^3)N-$, $-CON(R^3)-$, $-OC(O)N(R^3)-$, $-CSN(R^3)-$, $-N(R^3)CO-$, $-N(R^3)C(O)O-$, $-N(R^3)CS-$, $-S(O)_2N(R^3)-$, $-N(R^3)S(O)_2-$, $-N(R^3)CON(R^3)-$, $-N(R^3)CSN(R^3)-$ or $-N(R^3)SO_2N(R^3)-$ groups. Where L^2 contains two R^3 groups these may be the same or different.

The optional substituents which may be present on aliphatic or heteroaliphatic chains represented by Alk^1 include one, two, three or more substituents where each substituent may be the same or different and is selected from halogen atoms, e.g. fluorine, chlorine, bromine or iodine atoms, or -OH, $-CO_2H$, $-CO_2R^4$ [where R^4 is an optionally substituted straight or branched C_{1-6}alkyl group], e.g. $-CO_2CH_3$ or $-CO_2C(CH_3)_3$, $-CONHR^4$, e.g. $-CONHCH_3$, $-CON(R^4)_2$, e.g. $-CON(CH_3)_2$, $-COR^4$, e.g. $-COCH_3$, C_{1-6}alkoxy, e.g. methoxy or ethoxy, haloC_{1-6}alkoxy, e.g. trifluoromethoxy or difluoromethoxy, thiol (-SH), $-S(O)R^4$, e.g. $-S(O)CH_3$, $-S(O)_2R^4$, e.g. $-S(O)_2CH_3$, C_{1-6}alkylthio e.g. methylthio or ethylthio, amino, $-NHR^4$, e.g. $-NHCH_3$ or $-N(R^4)_2$, e.g. $-N(CH_3)_2$ groups. Where two R^4

groups are present in any of the above substituents these may be the same or different.

In addition when two R^4 alkyl groups are present in any of the optional substituents just described these groups may be joined, together with the N atom to which they are attached, to form a heterocyclic ring. Such heterocyclic rings may be optionally interrupted by a further heteroatom or heteroatom containing group selected from –O-, -S-, –N(R^4)-, -C(O)- or –C(S)- groups. Particular examples of such heterocyclic rings include piperidinyl, pyrazolidinyl, morpholinyl, thiomorpholinyl, pyrrolidinyl, imidazolidinyl and piperazinyl rings.

When L^1 is present in compounds of formula (1) as a linker atom or group it may be any such atom or group as hereinbefore described in relation to L^2 linker atoms and groups.

Optionally substituted cycloaliphatic groups represented by the group Cy^1 in compounds of the invention include optionally substituted C_{3-10}cycloaliphatic groups. Particular examples include optionally substituted C_{3-10}cycloalkyl, e.g. C_{3-7}cycloalkyl or C_{3-10}cycloalkenyl, e.g C_{3-7}cycloalkenyl groups.

Optionally substituted heterocycloaliphatic groups represented by the group Cy^1 include optionally substituted C_{3-10}heterocycloaliphatic groups. Particular examples include optionally substituted C_{3-10}heterocycloalkyl, e.g. C_{3-7}heterocycloalkyl or C_{3-10}heterocycloalkenyl, e.g. C_{3-7}heterocycloalkenyl groups, each of said groups containing one, two, three or four heteroatoms or heteroatom containing groups L^4 in place of or in addition to the ring carbon atoms where L^4 is an atom or group as previously defined for L^2.

Optionally substituted polycycloaliphatic groups represented by the group Cy^1 include optionally substituted C_{7-10}bi-or tricycloalkyl or C_{7-10}bi- or

tricycloalkenyl groups. Optionally substituted heteropolycycloaliphatic groups represented by the group Cy^1 include optionally substituted C_{7-10}bi- or tricycloalkyl or C_{7-10}bi- or tri-cycloalkenyl groups containing one, two, three, four or more L^4 atoms or groups in place of or in addition to the ring carbon atoms.

Particular examples of cycloaliphatic, polycycloaliphatic, heterocycloaliphatic and heteropolycycloaliphatic groups represented by the group Cy^1 include optionally substituted cyclopropyl, cyclobutyl, cyclopentyl, cyclohexyl, cycloheptyl, 2-cyclobuten-1-yl, 2-cyclopenten-1-yl, 3-cyclopenten-1-yl, adamantyl, norbornyl, norbornenyl, dihydrofuranyl, tetrahydrofuranyl, tetrahydropyranyl, dihydrothiophenyl, tetrahydrothiophenyl, pyrroline, e.g. 2- or 3-pyrrolinyl, pyrrolidinyl, pyrrolidinone, oxazolidinyl, oxazolidinone, dioxolanyl, e.g. 1,3-dioxolanyl, imidazolinyl, e.g. 2-imidazolinyl, imidazolidinyl, pyrazolinyl, e.g. 2-pyrazolinyl, pyrazolidinyl, 5,6-dihydro-2(1H)-pyrazinone, tetrahydropyrimidinyl, thiazolinyl, thiazolidinyl, pyranyl, e.g. 2- or 4-pyranyl, piperidinyl, homopiperidinyl, heptamethyleneiminyl, piperidinone, 1,4-dioxanyl, morpholinyl, morpholinone, 1,4-dithianyl, thiomorpholinyl, piperazinyl, homopiperazinyl, 1,3,5-trithianyl, oxazinyl, e.g. 2H-1,3-, 6H-1,3-, 6H-1,2-, 2H-1,2- or 4H-1,4-oxazinyl, 1,2,5-oxathiazinyl, isoxazinyl, e.g. o- or p-isoxazinyl, oxathiazinyl, e.g. 1,2,5 or 1,2,6-oxathiazinyl, 1,3,5-oxadiazinyl, dihydroisothiazolyl, dihydroisothiazole 1,1-dioxide, e.g. 2,3-dihydroisothiazole 1,1-dioxide, dihydropyrazinyl and tetrahydropyrazinyl groups.

The optional substituents which may be present on the cycloaliphatic, polycycloaliphatic, heterocycloaliphatic or heteropolycycloaliphatic groups represented by the group Cy^1 include one, two, three or more substituents selected from halogen atoms, or C_{1-6}alkyl, e.g. methyl or ethyl, haloC_{1-6}alkyl, e.g. halomethyl or haloethyl such as difluoromethyl or trifluoromethyl, optionally substituted by hydroxyl, e.g. $-C(OH)(CF_3)_2$, C_{1-6}alkoxy, e.g.

methoxy or ethoxy, haloC_{1-6}alkoxy, eg. halomethoxy or haloethoxy such as difluoromethoxy or trifluoromethoxy, thiol, C_{1-6}alkylthiol, e.g. methylthiol or ethylthiol, carbonyl (=O), thiocarbonyl (=S), imino (=NR^{4a}) [where R^{4a} is an –OH group or a C_{1-6}alkyl group], or $-(Alk^3)_vR^5$ groups in which Alk^3 is a straight or branched C_{1-3}alkylene chain, v is zero or the integer 1 and R^5 is a C_{3-8}cycloalkyl, –OH, -SH, -$N(R^6)(R^7)$ [in which R^6 and R^7 is each independently selected from a hydrogen atom or an optionally substituted alkyl or C_{3-8}cycloalkyl group], -OR^6, -SR^6, -CN, -NO_2, -CO_2R^6, -SOR^6, -SO_2R^6, -SO_3R^6, -OCO_2R^6, -$C(O)R^6$, -$OC(O)R^6$, -$C(S)R^6$, -$C(O)N(R^6)(R^7)$, -$OC(O)N(R^6)(R^7)$, -$N(R^6)C(O)R^7$, -$C(S)N(R^6)(R^7)$, -$N(R^6)C(S)R^7$, -$SO_2N(R^6)(R^7)$, -$N(R^6)SO_2R^7$, -$N(R^6)C(O)N(R^7)(R^8)$ [where R^8 is as defined for R^6], -$N(R^6)C(S)N(R^7)(R^8)$, -$N(R^6)SO_2N(R^7)(R^8)$ or an optionally substituted aromatic or heteroaromatic group.

Particular examples of Alk^3 chains include $-CH_2-$, $-CH_2CH_2-$, $-CH_2CH_2CH_2-$ and $-CH(CH_3)CH_2-$ chains.

When R^5, R^6, R^7 and/or R^8 is present as a C_{3-8}cycloalkyl group it may be for example a cyclopropyl, cyclobutyl, cyclopentyl or cyclohexyl group. Optional substituents which may be present on such groups include for example one, two or three substituents which may be the same or different selected from halogen atoms, for example fluorine, chlorine, bromine or iodine atoms, or hydroxy or C_{1-6}alkoxy, e.g. methoxy, ethoxy or *i*-propoxy groups.

When the groups R^6 and R^7 or R^7 and R^8 are both alkyl groups these groups may be joined, together with the N atom to which they are attached, to form a heterocyclic ring. Such heterocyclic rings may be optionally interrupted by a further heteroatom or heteroatom containing group selected from –O-, -S-, –$N(R^7)$-, -C(O)- or –C(S)- groups. Particular examples of such heterocyclic rings include piperidinyl, pyrazolidinyl, morpholinyl, thiomorpholinyl, pyrrolidinyl, imidazolidinyl and piperazinyl rings.

When R^5 is an optionally substituted aromatic or heteroaromatic group it may be any such group as described hereinafter in relation to Cy^1.

Additionally, when the group Cy^1 is a heterocycloaliphatic or heteropolycycloaliphatic group containing one or more nitrogen atoms each nitrogen atom may be optionally substituted by a group $-(L^5)_p(Alk^4)_qR^9$ in which L^5 is a $-C(O)-$, $-C(O)O-$, $-C(S)-$, $-S(O)_2-$, $-CON(R^6)-$ or $-SO_2N(R^6)-$ group; p is zero or the integer 1; Alk^4 is an optionally substituted aliphatic or heteroaliphatic chain; q is zero or the integer 1; and R^9 is a hydrogen atom or an optionally substituted cycloaliphatic, heterocycloaliphatic, polycycloaliphatic, heteropolycycloaliphatic, aromatic or heteroaromatic group as herein described in relation to Cy^1.

When Alk^4 is present as an aliphatic or heteroaliphatic chain it may be for example any aliphatic or heteroaliphatic chain as hereinbefore described for Alk^1.

Optionally substituted aromatic groups represented by the groups Cy^1 include for example monocyclic or bicyclic fused ring C_{6-12}aromatic groups, such as phenyl, 1- or 2-napthyl, 1- or 2-tetrahydronapthyl, indanyl or indenyl groups.

Heteroaromatic groups represented by the groups Cy^1 include for example C_{1-9}heteroaromatic groups containing for example one, two, three or four heteroatoms selected from oxygen, sulphur or nitrogen atoms. In general, the heteroaromatic groups may be for example monocyclic or bicyclic fused ring heteroaromatic groups. Monocyclic heteroaromatic groups include for example five- or six-membered heteroaromatic groups containing one, two, three or four heteroatoms selected from oxygen, sulphur or nitrogen atoms. Bicyclic heteroaromatic groups include for example eight- to thirteen-

membered fused ring heteroaromatic groups containing one, two or more heteroatoms selected from oxygen, sulphur or nitrogen atoms.

Particular examples of heteroaromatic groups of these types include pyrrolyl, furyl, thienyl, imidazolyl, N-C_{1-6}alkylimidazolyl, oxazolyl, isoxazolyl, thiazolyl, isothiazolyl, pyrazolyl, 1,2,3-triazolyl, 1,2,4-triazolyl, 1,2,3-oxadiazolyl, 1,2,5-oxadiazolyl, 1,3,4-oxadiazolyl, 1,3,4-thiadiazolyl, pyridyl, pyrimidinyl, pyridazinyl, pyrazinyl, 1,3,5-triazinyl, 1,2,4-triazinyl, 1,2,3-triazinyl, benzofuryl, [2,3-dihydro]benzofuryl, benzothienyl, [2,3-dihydro]benzothienyl, benzotriazolyl, indolyl, indolinyl, indazolinyl, benzimidazolyl, imidazo[1,2-a]pyridyl, benzothiazolyl, benzoxazolyl, benzisoxazolyl, benzopyranyl, [3,4-dihydro]benzopyranyl, quinazolinyl, quinoxalinyl, naphthyridinyl, imidazo[1,5-a]pyridinyl, imidazo[1,5-a]pyrazinyl, imidazo[1,5-c]pyrimidinyl, pyrido[3,4-b]pyridyl, pyrido[3,2-b]pyridyl, pyrido[4,3-b]pyridyl, quinolinyl, isoquinolinyl, phthalazinyl, tetrazolyl, 5,6,7,8-tetrahydroquinolinyl, 5,6,7,8-tetrahydroisoquinolinyl, imidyl, e.g. succinimidyl, phthalimidyl or naphthalimidyl such as 1,8-naphthalimidyl, pyrazolo[4,3-d]pyrimidinyl, furo[3,2-d]pyrimidinyl, thieno[3,2-d]pyrimidinyl, pyrrolo[3,2-d]pyrimidinyl, pyrazolo[3,2-b]pyridinyl, furo[3,2-b]pyridinyl, thieno[3,2-b]pyridinyl, pyrrolo[3,2-b]pyridinyl, thiazolo[3,2-a]pyyridinyl, pyrido[1,2-a]pyrimidinyl, tetrahydroimidazo[1,2-a]pyrimidinyl and dihydroimidazo[1,2-a]pyrimidinyl groups.

Optional substituents which may be present on aromatic or heteroaromatic groups represented by the group Cy^1 include one, two, three or more substituents, each selected from an atom or group R^{10} in which R^{10} is R^{10a} or $-L^6Alk^5(R^{10a})_r$, where R^{10a} is a halogen atom, or an amino ($-NH_2$), substituted amino, nitro, cyano, hydroxyl (-OH), substituted hydroxyl, formyl, carboxyl (-CO_2H), esterified carboxyl, thiol (-SH), substituted thiol, $-COR^{11}$ [where R^{11} is an $-L^6Alk^3(R^{10a})_r$, aryl or heteroaryl group], $-CSR^{11}$, $-SO_3H$, $-SOR^{11}$, $-SO_2R^{11}$, $-SO_3R^{11}$, $-SO_2NH_2$, $-SO_2NHR^{11}$, $-SO_2N(R^{11})_2$, $-CONH_2$, $-CSNH_2$, $-CONHR^{11}$, -

$CSNHR^{11}$, $-CON(R^{11})_2$, $-CSN(R^{11})_2$, $-N(R^{12})SO_2R^{11}$ [where R^{12} is a hydrogen atom or a straight or branched alkyl group], $-N(SO_2R^{11})_2$, $-N(R^{12})SO_2NH_2$, $-N(R^{12})SO_2NHR^{11}$, $-N(R^{12})SO_2N(R^{11})_2$, $-N(R^{12})COR^{11}$, $-N(R^{12})CONH_2$, $-N(R^{12})CONHR^{11}$, $-N(R^{12})CON(R^{11})_2$, $-N(R^{12})CSNH_2$, $-N(R^{12})CSNHR^{11}$, $-N(R^{12})CSN(R^{11})_2$, $-N(R^{12})CSR^{11}$, $-N(R^{12})C(O)OR^{11}$, $-SO_2NHet^1$ [where $-NHet^1$ is an optionally substituted C_{5-7}cyclicamino group optionally containing one or more other –O- or -S- atoms or $-N(R^{12})-$, -C(O)- or –C(S)- groups], $-CONHet^1$, $-CSNHet^1$, $-N(R^{12})SO_2NHet^1$, $-N(R^{12})CONHet^1$, $-N(R^{12})CSNHet^1$, $-SO_2N(R^{12})Het$ [where -Het is an optionally substituted monocyclic C_{5-7}carbocyclic group optionally containing one or more other –O- or –S- atoms or $-N(R^{12})-$, -C(O)-, -S(O)- or $-S(O)_2-$ groups], -Het, $-CON(R^{12})Het$, $-CSN(R^{12})Het$, $-N(R^{12})CON(R^{12})Het$, $-N(R^{12})CSN(R^{12})Het$, $-N(R^{12})SO_2N(R^{12})Het$, aryl or heteroaryl groups; L^6 is a covalent bond or a linker atom or group as hereinbefore defined for L^2; Alk^5 is an optionally substituted straight or branched C_{1-6}alkylene, C_{2-6}alkenylene or C_{2-6}alkynylene chain, optionally interrupted by one, two or three –O- or -S- atoms or $-S(O)_n-$ [where n is an integer 1 or 2] or $-N(R^{12})-$ e.g. $-N(CH_3)-$ groups; and r is zero or the integer 1, 2, or 3. It will be appreciated that when two R^{11} or R^{12} groups are present in one of the above substituents the R^{11} and R^{12} groups may be the same or different.

When in the group $-L^6Alk^5(R^{10a})_r$ r is an integer 1, 2 or 3, it is to be understood that the substituent or substituents R^{10a} may be present on any suitable carbon atom in $-Alk^5$. Where more than one R^{10a} substituent is present these may be the same or different and may be present on the same or different atom in $-Alk^5$. Clearly, when r is zero and no substituent R^{10a} is present the alkylene, alkenylene or alkynylene chain represented by Alk^5 becomes an alkyl, alkenyl or alkynyl group.

When R^{10a} is a substituted amino group it may be for example a group $-NHR^{11}$ [where R^{11} is as defined above] or a group $-N(R^{11})_2$ wherein each R^{11} group is the same or different.

When R^{10a} is a halogen atom it may be for example a fluorine, chlorine, bromine, or iodine atom.

When R^{10a} is a substituted hydroxyl or substituted thiol group it may be for example a group $-OR^{11}$ or a $-SR^{12}$ group respectively.

Esterified carboxyl groups represented by the group R^{10a} include groups of formula $-CO_2Alk^6$ wherein Alk^6 is a straight or branched, optionally substituted C_{1-8}alkyl group such as a methyl, ethyl, n-propyl, i-propyl, n-butyl, i-butyl, s-butyl or t-butyl group; a $C_{6-12}arylC_{1-8}$alkyl group such as an optionally substituted benzyl, phenylethyl, phenylpropyl, 1-naphthylmethyl or 2-naphthylmethyl group; a C_{6-12}aryl group such as an optionally substituted phenyl, 1-naphthyl or 2-naphthyl group; a $C_{6-12}aryloxyC_{1-8}$alkyl group such as an optionally substituted phenyloxymethyl, phenyloxyethyl, 1-naphthyloxymethyl, or 2-naphthyloxymethyl group; an optionally substituted $C_{1-8}alkanoyloxyC_{1-8}$alkyl group, such as a pivaloyloxymethyl, propionyloxyethyl or propionyloxypropyl group; or a $C_{6-12}aroyloxyC_{1-8}$alkyl group such as an optionally substituted benzoyloxyethyl or benzoyloxypropyl group. Optional substituents present on the Alk^6 group include R^{10a} atoms and groups as described above.

When Alk^5 is present in or as a substituent it may be for example a $-CH_2-$, $-CH(CH_3)-$, $-C(CH_3)_2-$, $-CH_2CH_2-$, $-CH_2CH_2CH_2-$, $-CH(CH_3)CH_2-$, $-CH_2CH_2CH_2CH_2-$, $-CH_2CH(CH_3)CH_2-$, $-CH(CH_3)CH_2CH_2-$, $-C(CH_3)_2CH_2-$, $-CH=CH-$, $-CH=CCH_2-$, $-CH_2C=CH-$, $-CH=CHCH_2CH_2-$, $-CH_2CH=CHCH_2-$, $-CH_2CH_2CH=CH_2-$, $-CC-$, $-CCCH_2-$, $-CH_2CC-$, $-CCCH_2CH_2-$, $-CH_2CCCH_2-$ or $-CH_2CH_2CC-$ chain, optionally interrupted by one, two, or three -O- or -S-, atoms or $-S(O)-$, $-S(O)_2-$ or $-N(R^{12})-$, e.g. $-N(CH_3)-$ groups. The aliphatic chains

represented by Alk^5 may be optionally substituted by one, two or three halogen atoms in addition to any R^{10a} groups that may be present.

Aryl or heteroaryl groups represented by the groups R^{10a} or R^{11} include mono- or bicyclic optionally substituted C_{6-12} aromatic or C_{1-9} heteroaromatic groups as described above for the group Cy^1. The aromatic and heteroaromatic groups may be attached to the group Cy^1 in compounds of formula (1) by any carbon or hetero e.g. nitrogen atom as appropriate.

It will be appreciated that when $-NHet^1$ or –Het forms part of a substituent R^{10} the heteroatoms or heteroatom containing groups that may be present within the ring $-NHet^1$ or –Het take the place of carbon atoms within the parent carbocyclic ring.

Thus when $-NHet^1$ or –Het forms part of a substituent R^{10} each may be for example an optionally substituted pyrrolidinyl, imidazolidinyl, pyrazolidinyl, piperazinyl, morpholinyl, thiomorpholinyl, piperidinyl or thiazolidinyl group. Additionally Het may represent for example, an optionally substituted cyclopentyl or cyclohexyl group. Optional substituents which may be present on $-NHet^1$ include those substituents described above when Cy^1 is a heterocycloaliphatic group.

Particularly useful atoms or groups represented by R^{10} include fluorine, chlorine, bromine or iodine atoms, or C_{1-6}alkyl, e.g. methyl, ethyl, n-propyl, i-propyl, n-butyl or t-butyl, optionally substituted phenyl, pyridyl, pyrimidinyl, pyrrolyl, furyl, thiazolyl, or thienyl, C_{1-6}hydroxyalkyl, e.g. hydroxymethyl or hydroxyethyl, carboxyC_{1-6}alkyl, e.g. carboxyethyl, C_{1-6}alkylthio e.g. methylthio or ethylthio, carboxyC_{1-6}alkylthio, e.g. carboxymethylthio, 2-carboxyethylthio or 3-carboxy-propylthio, C_{1-6}alkoxy, e.g. methoxy or ethoxy, hydroxyC_{1-6}alkoxy, e.g. 2-hydroxyethoxy, optionally substituted phenoxy, pyridyloxy, thiazolyoxy, phenylthio or pyridylthio, C_{3-7}cycloalkyl, e.g. cyclobutyl, cyclopentyl, C_{5-}

$_{7}$cycloalkoxy, e.g. cyclopentyloxy, haloC_{1-6}alkyl, e.g. trifluoromethyl, haloC_{1-6}alkoxy, e.g. trifluoromethoxy, C_{1-6}alkylamino, e.g. methylamino, ethylamino, $-CH(CH_3)NH_2$ or $-C(CH_3)_2NH_2$, haloC_{1-6}alkylamino, e.g. fluoroC_{1-6}alkylamino, e.g. $-CH(CF_3)NH_2$ or $-C(CF_3)_2NH_2$, amino ($-NH_2$), aminoC_{1-6}alkyl, e.g. aminomethyl or aminoethyl, C_{1-6}dialkylamino, e.g. dimethylamino or diethylamino, C_{1-6}alkylaminoC_{1-6}alkyl, e.g. ethylaminoethyl, C_{1-6}dialkylaminoC_{1-6}alkyl, e.g. diethylaminoethyl, aminoC_{1-6}alkoxy, e.g. aminoethoxy, C_{1-6}alkylaminoC_{1-6}alkoxy, e.g. methylaminoethoxy, C_{1-6}dialkylaminoC_{1-6}alkoxy, e.g. dimethylaminoethoxy, diethylaminoethoxy, diisopropylaminoethoxy, or dimethylaminopropoxy, imido, such as phthalimido or naphthalimido, e.g. 1,8-naphthalimido, nitro, cyano, hydroxyl (-OH), formyl [HC(O)-], carboxyl ($-CO_2H$), $-CO_2Alk^6$ [where Alk^6 is as defined above], C_{1-6} alkanoyl e.g. acetyl, optionally substituted benzoyl, thiol (-SH), thioC_{1-6}alkyl, e.g. thiomethyl or thioethyl, sulphonyl ($-SO_3H$), C_{1-6}alkylsulphonyl, e.g. methylsulphonyl, aminosulphonyl ($-SO_2NH_2$), C_{1-6}alkylaminosulphonyl, e.g. methylaminosulphonyl or ethylaminosulphonyl, C_{1-6}dialkylaminosulphonyl, e.g. dimethylaminosulphonyl or diethylaminosulphonyl, phenylaminosulphonyl, carboxamido ($-CONH_2$), C_{1-6}alkylaminocarbonyl, e.g. methylaminocarbonyl or ethylaminocarbonyl, C_{1-6}dialkylaminocarbonyl, e.g. dimethylaminocarbonyl or diethylaminocarbonyl, aminoC_{1-6}alkylaminocarbonyl, e.g. aminoethylamino-carbonyl, C_{1-6}dialkylaminoC_{1-6}alkylaminocarbonyl, e.g. diethylaminoethyl-aminocarbonyl, aminocarbonylamino, C_{1-6}alkylaminocarbonylamino, e.g. methylaminocarbonylamino or ethylaminocarbonylamino, C_{1-6}dialkylamino-carbonylamino, e.g. dimethylaminocarbonylamino or diethylamino-carbonylamino, C_{1-6}alkylaminocabonylC_{1-6}alkylamino, e.g. methylamino-carbonylmethylamino, aminothiocarbonylamino, C_{1-6}alkylaminothiocarbonyl-amino, e.g. methylaminothiocarbonylamino or ethylaminothiocarbonylamino, C_{1-6}dialkylaminothiocarbonylamino, e.g. dimethylaminothiocarbonylamino or diethylaminothiocarbonylamino, C_{1-6}alkylaminothiocarbonylC_{1-6}alkylamino, e.g. ethylaminothiocarbonylmethylamino, $-CONHC(=NH)NH_2$, C_{1-6}alkylsulphonyl-amino, e.g. methylsulphonylamino or ethylsulphonylamino, C_{1-6}dialkyl-

sulphonylamino, e.g. dimethylsulphonylamino or diethylsulphonylamino, optionally substituted phenylsulphonylamino, aminosulphonylamino (-$NHSO_2NH_2$), C_{1-6}alkylaminosulphonylamino, e.g. methylaminosulphonylamino or ethylaminosulphonylamino, C_{1-6}dialkylaminosulphonylamino, e.g. dimethylaminosulphonylamino or diethylaminosulphonylamino, optionally substituted morpholinesulphonylamino or morpholinesulphonylC_{1-6}alkylamino, optionally substituted phenylaminosulphonylamino, C_{1-6}alkanoylamino, e.g. acetylamino, aminoC_{1-6}alkanoylamino e.g. aminoacetylamino, C_{1-6}dialkylaminoC_{1-6}alkanoylamino, e.g. dimethylaminoacetylamino, C_{1-6}alkanoylaminoC_{1-6}alkyl, e.g. acetylaminomethyl, C_{1-6}alkanoylaminoC_{1-6}alkylamino, e.g. acetamidoethylamino, C_{1-6}alkoxycarbonylamino, e.g. methoxycarbonylamino, ethoxycarbonylamino or t-butoxycarbonylamino or optionally substituted benzyloxy, pyridylmethoxy, thiazolylmethoxy, benzyloxycarbonylamino, benzyloxycarbonylaminoC_{1-6}alkyl e.g. benzyloxycarbonylaminoethyl, benzothio, pyridylmethylthio or thiazolylmethylthio groups.

A further particularly useful group of substituents represented by R^{10} when present on aromatic or heteroaromatic groups includes substituents of formula – $L^6Alk^5R^{10a}$ where L^6 is preferably a covalent bond or an –O- or -S- atom or –$N(R^3)$-, -C(O)-, -C(O)O-, -O-C(O)-, -$N(R^3)$CO-, -$CON(R^3)$- or –$N(R^3)S(O)_2$- group, Alk^5 is an optionally substituted C_{1-6}alkyl group optionally interrupted by one or two –O- or –S- atoms or –$N(R^{12})$-, -C(O)-, -C(S)-, -$CON(R^{12})$- or –$N(R^{12})$CO- groups and R^{10a} is an optionally substituted Het group as herein defined or an optionally substituted heteroaromatic group as hereinbefore described in relation to Cy^1.

Where desired, two R^{10} substituents may be linked together to form a cyclic group such as a cyclic ether, e.g. a C_{1-6}alkylenedioxy group such as methylenedioxy or ethylenedioxy.

It will be appreciated that where two or more R^{10} substituents are present, these need not necessarily be the same atoms and/or groups. In general, the substituent(s) may be present at any available ring position on the aromatic or heteroaromatic group represented by the group Cy^1.

The substituted aromatic or heteroaromatic group represented by Ar in compounds of the invention may be any aromatic or heteroaromatic group as hereinbefore described for Cy^1. Optional substituents which may be present include those R^{10} atoms and groups as generally or particularly described in relation to Cy^1 aromatic and heteroaromatic groups.

In compounds of formula (1) R is preferably a hydrogen atom.

In compounds of this type and in general in compounds of formula (1) X is preferably an –O- or –S- atom, and is especially a –S- atom.

In general in compounds of formula (1) R^a is preferably a hydrogen atom or a C_{1-4}alkyl group, especially a methyl, ethyl, n-propyl or i-propyl group. In particular R^a is a methyl group or more especially a hydrogen atom.

In another particular class of compounds of formula (1) the bond represented by the dashed line is present and A is a $-C(R^b)=$ group. In these compounds R^b is preferably a hydrogen atom or a C_{1-4}alkyl group, especially a methyl, ethyl, n-propyl or i-propyl group. More particularly R^b is a methyl group or more especially a hydrogen atom.

When in compounds of formula (1) n is the integer 1, Alk^1 is preferably an optionally substituted C_{1-6}alkylene chain, especially an optionally substituted $-CH_2-$, $-CH_2CH_2-$, $-CH_2CH_2CH_2-$, $-CH(CH_3)CH_2-$ or $-CH_2CH(CH_3)-$ chain, most especially a $-CH_2-$ or $-CH_2CH_2-$ chain.

In one class of compounds of formula (1) n is zero.

The group L^1 in compounds of formula (1) is preferably a covalent bond or an –O- or –S- atom or an $-N(R^3)-$, especially –NH- or $-N(CH_3)-$, -C(O)-, -C(S)-, -S(O)- or $-S(O)_2-$ group. More particularly L^1 is a covalent bond or an –O- or –S- atom or –NH- group. L^1 is more especially preferably is a covalent bond.

Cy^1 in compounds of formula (1) is preferably an optionally substituted cycloaliphatic, aromatic or heteroaromatic group as hereinbefore generally and particularly defined.

Particularly preferred Cy^1 optionally substituted cycloaliphatic groups include optionally substituted C_{3-7}cycloalkyl groups, especially cyclopropyl, cyclobutyl, cyclopentyl or cyclohexyl groups.

Particularly preferred optional substituents which may be present on Cy^1 optionally substituted cycloaliphatic groups include halogen atoms, especially fluorine, chlorine or bromine atoms, or C_{1-6}alkyl groups, especially C_{1-3}alkyl groups, most especially a methyl group, or a haloC_{1-6}alkyl group, especially a fluoroC_{1-6}alkyl group, most especially a $-CF_3$ group, or a C_{1-6}alkoxy, especially methoxy, ethoxy, propxy or i-propoxy group, or a haloC_{1-6}alkoxy, especially a fluoroC_{1-6}alkoxy, most especially a $-OCF_3$ group, or a cyano (-CN), esterified carboxyl, especially $-CO_2CH_3$ or $-CO_2C(CH_3)_3$, nitro ($-NO_2$), amino ($-NH_2$), substituted amino, especially $-NHCH_3$ or $-N(CH_3)_2$, $-C(O)R^6$, especially $-C(O)CH_3$, or $-N(R^6)C(O)R^7$, especially $-NHCOCH_3$ group.

Particularly preferred Cy^1 aromatic groups include optionally substituted phenyl groups. Particularly preferred heteroaromatic groups include optionally substituted monocyclic heteroaromatic groups, especially optionally substituted five- or six-membered heteroaromatic groups containing one, two, three or four heteroatoms selected from oxygen, sulphur

or nitrogen atoms. Particularly preferred optionally substituted monocyclic heteroaromatic groups include optionally substituted furyl, thienyl, pyrrolyl, oxazolyl, thiazolyl, pyridyl, pyrimidinyl or triazinyl group.

Particularly preferred optional substituents which may be present on Cy^1 aromatic or heteroaromatic groups include atoms or groups $-R^{10a}$ or $-L^6Alk^5(R^{10a})_r$ as hereinbefore defined. Particularly useful optional substituents include halogen atoms, especially fluorine, chlorine or bromine atoms, or C_{1-6}alkyl groups, especially C_{1-3}alkyl groups, most especially a methyl group, or a haloC_{1-6}alkyl group, especially a fluoroC_{1-6}alkyl group, most especially a $-CF_3$ group, or a C_{1-6}alkoxy, especially methoxy, ethoxy, propxy or i-propoxy group, or a haloC_{1-6}alkoxy, especially a fluoroC_{1-6}alkoxy, most especially a $-OCF_3$ group, or a cyano (-CN), carboxyl ($-CO_2H$), esterified carboxyl ($-CO_2Alk^6$), especially $-CO_2CH_3$, $-CO_2CH_2CH_3$, or $-CO_2C(CH_3)_3$, nitro ($-NO_2$), amino ($-NH_2$), substituted amino, especially $-NHCH_3$ or $-N(CH_3)_2$, $-COR^{11}$, especially $-COCH_3$, or $-N(R^{12})COR^{11}$, especially $-NHCOCH_3$ group.

Further preferred optional substituents which may be present on Cy^1 aromatic or heteroaromatic groups include groups of formula $-L^6Alk^5(R^{10a})_r$ in which r is the integer 1, L^6 is a covalent bond or an –O- or –S- atom or a $-N(R^3)-$, especially –NH- or $-N(CH_3)-$, -C(O)-, -C(S)-, -C(O)O-, -OC(O)-, $-N(R^3)CO-$, especially –NHCO-, or $-CON(R^3)-$, especially –CHNH-group, Alk^5 is a C_{1-6}alkyl chain, especially a $-CH_2-$, $-CH_2CH_2-$, $-CH_2CH_2CH_2-$ or $-CH_2CH_2CH_2CH_2-$ chain and R^{10a} is a substituted hydroxyl group, especially a $-OCH_3$, $-OCH_2CH_3$ or $-OCH(CH_3)_2$ group or a substituted amino group, especially a $-N(CH_3)_2$ or $-N(CH_2CH_3)_2$ group or a –Het group, especially an optionally substituted monocyclic C_{5-7}carbocyclic group containing one, two or three –O-, -S-, $-N(R^{12})-$, especially –NH- or $-N(CH_3)-$or –C(O)- groups within the ring structure as previously described, most especially an optionally substituted pyrrolidinyl, imidazolidinyl, piperidinyl, e.g. N-methylpiperidinyl, morpholinyl, thiomorpholinyl or piperazinyl group or R^{10a} is

an optionally substituted heteroaromatic group, especially a five- or six-membered monocyclic heteroaromatic group containing one, two, three or four heteroatoms selected from oxygen, sulphur or nitrogen atoms, such as optionally substituted pyrrolyl, furyl, thienyl, imidazolyl, triazolyl, pyridyl, pyrimidinyl, triazinyl, pyridazinyl, or pyrazinyl group. Particularly preferred optional substituents on the –Het groups just described include hydroxyl (-OH) and carboxyl (-CO_2H) groups or those preferred optional substituents just described in relation to the group Cy^1.

In one particularly preferred group of compounds of formula (1) Cy^1 is an optionally substituted phenyl group, especially a phenyl group optionally substituted by one, two or three optional substituents where at least one, and preferably two optional substituents are located *ortho* to the bond joining Cy^1 to the remainder of the compound of formula (1), (1a) or (2a). Particularly preferred *ortho* substituents include halogen atoms, especially fluorine or chlorine atoms, or C_{1-3}alkyl groups, especially methyl groups, C_{1-3}alkoxy groups, especially methoxy, haloC_{1-3}alkyl groups, especially -CF_3, haloC_{1-3}alkoxy groups, especially –OCF_3, or cyano (-CN), groups. In this class of compounds a second or third optional substituent when present in a position other than the *ortho* positions of the ring Cy^1 may be preferably an atom or group $–R^{10a}$ or $–L^6Alk^5(R^{10a})_r$ as herein generally and particularly described.

The group Y in compounds of formula (1) is preferably a –CH= group or a substituted carbon atom. Particular substituted carbon atoms include those where Y is $–C(R^{10})=$ wherein R^{10} is as generally or particularly described above, especially those $-R^{10a}$ and $–L^6Alk^5(R^{10a})_r$ substituents just described with respect to those preferred optional substituents present on Cy^1 aromatic or heteroaromatic groups. Particularly useful compounds of formula (1) are those compounds wherein Y is –CH= or $–C(R^{10})=$ in which R^{10} is a –CN, -$CONH_2$, -$CONHR^{11}$, -$CON(R^{11})_2$, -$CONHet^1$, -$CON(R^{12})$Het, -$CON(R^{12})Alk^5$Het, or esterified carboxyl, particularly $–CO_2Alk^6$ group as generally or particularly described herein.

Particularly preferred Ar aromatic groups in compounds of formula (1) include optionally substituted phenyl groups. Particularly preferred heteroaromatic groups include optionally substituted monocyclic heteroaromatic groups, especially optionally substituted five- or six-membered heteroaromatic groups containing one, two, three or four heteroatoms selected from oxygen, sulphur or nitrogen atoms. Particularly preferred optionally substituted monocyclic heteroaromatic groups include optionally substituted furyl, thienyl, pyrrolyl, oxazolyl, thiazolyl, pyridyl, pyrimidinyl or triazinyl group.

Particularly preferred optional substituents which may be present on Ar aromatic or heteroaromatic groups include atoms or groups $-R^{10a}$ or $-L^6Alk^5(R^{10a})_r$ as hereinbefore defined. Particularly useful optional substituents include halogen atoms, especially fluorine, chlorine or bromine atoms, or C_{1-6}alkyl groups, especially C_{1-3}alkyl groups, most especially a methyl group, or a haloC_{1-6}alkyl group, especially a fluoroC_{1-6}alkyl group, most especially a $-CF_3$ group, or a C_{1-6}alkoxy, especially methoxy, ethoxy, propxy or i-propoxy group, or a haloC_{1-6}alkoxy, especially a fluoroC_{1-6}alkoxy, most especially a $-OCF_3$ group, or a cyano (-CN), esterified carboxyl, especially $-CO_2CH_3$ or $-CO_2C(CH_3)_3$, nitro ($-NO_2$), amino ($-NH_2$), substituted amino, especially $-NHCH_3$ or $-N(CH_3)_2$, $-COR^{11}$, especially $-COCH_3$, or $-N(R^{12})COR^{11}$, especially $-NHCOCH_3$ group.

Particularly useful Ar groups in compounds of formula (1) include phenyl and mono- or disubstituted phenyl groups in which each substituent is in particular a $-R^{10a}$ or $-L^6Alk^5(R^{10a})_r$ atom or group as just defined and is especially a halogen atom or a C_{1-3}alkyl, C_{1-3}alkoxy or –CN group

Particularly useful compounds of the invention include each of the compounds described in the Examples hereinafter, and the salts, solvates, hydrates and N-oxides thereof.

Compounds according to the invention are potent and selective inhibitors of p38 kinases, including all isoforms and splice variants thereof. More specifically the compounds of the invention are inhibitors of p38α, p38β and p38β2. The ability of the compounds to act in this way may be simply determined by employing tests such as those described in the Examples hereinafter.

The compounds of formula (1) are of use in modulating the activity of p38 kinases and in particular are of use in the prophylaxis and treatment of any p38 kinase mediated diseases or disorders in a human, or other mammal. The invention extends to such a use and to the use of the compounds for the manufacture of a medicament for treating such diseases or disorders. Further the invention extends to the administration to a human an effective amount of a p38 inhibitor for treating any such disease or disorder.

The invention also extends to the prophylaxis or treatment of any disease or disorder in which p38 kinase plays a role including conditions caused by excessive or unregulated pro-inflammatory cytokine production including for example excessive or unregulated TNF, IL-1, IL-6 and IL-8 production in a human, or other mammal. The invention extends to such a use and to the use of the compounds for the manufacture of a medicament for treating such cytokine-mediated diseases or disorders. Further the invention extends to the administration to a human an effective amount of a p38 inhibitor for treating any such disease or disorder.

Diseases or disorders in which p38 kinase plays a role either directly or via pro-inflammatory cytokines including the cytokines TNF, IL-1, IL-6 and IL-8 include without limitation autoimmune diseases, inflammatory diseases, destructive-bone disorders, proliferative disorders, neurodegenerative disorders, viral diseases, allergies, infectious diseases, heart attacks,

angiogenic disorders, reperfusion/ischemia in stroke, vascular hyperplasia, organ hypoxia, cardiac hypertrophy, thrombin-induced platelet aggregation and conditions associated with prostaglandin endoperoxidase synthetase-2 (COX-2).

Autoimmune diseases which may be prevented or treated include but are not limited to rheumatoid arthritis, inflammatory bowel disease, ulcerative colitis, Crohn's disease, multiple sclerosis, diabetes, glomerulonephritis, systemic lupus erythematosus, scleroderma, chronic thyroiditis, Grave's disease, hemolytic anemia, autoimmune gastritis, autoimmune neutropenia, thrombocytopenia, chronic active hepatitis, myasthenia gravis, atopic dermatitis, graft vs, host disease or psoriasis.

The invention further extends to the particular autoimmune disease rheumatoid arthritis.

Inflammatory diseases which may be prevented or treated include but are not limited to asthma, allergies, respiratory distress syndrome or acute or chronic pancreatitis.

Destructive bone disorders which may be prevented or treated include but are not limited to osteoporosis, osteoarthritis and multiple myeloma-related bone disorder.

Proliferative diseases which may be prevented or treated include but are not limited to acute or chronic myelogenous leukemia, Kaposi's sarcoma, metastic melanoma and multiple myeloma.

Neurodegenerative diseases which may be prevented or treated include but are not limited to Parkinson's disease, Alzheimer's disease, cerebral ischemias or neurodegenerative disease caused by traumatic injury.

Viral diseases which may be prevented or treated include but are not limited to acute hepatitis infection (including hepatitis A, hepatitis B and hepatitis C), HIV infection and CMV retinitis.

Infectious diseases which may be prevented or treated include but are not limited to septic shock, sepsis and Shigellosis.

In addition, p38 inhibitors of this invention also exhibit inhibition of expression of inducible pro-inflammatory proteins such as prostaglandin endoperoxidase synthetase-2, otherwise known as cyclooxygenase-2 (COX-2) and are therefore of use in therapy. Pro-inflammatory mediators of the cyclooxygenase pathway derived from arachidonic acid are produced by inducible COX-2 enzyme. Regulation of COX-2 would regulate these pro-inflammatory mediators such as prostaglandins, which affect a wide variety of cells and are important and critical inflammatory mediators of a wide variety of disease states and conditions. In particular these inflammatory mediators have been implicated in pain, such as in the sensitization of pain receptors, or edema. Accordingly additional p38 mediated conditions which may be prevented or treated include edema, analgesia, fever and pain such as neuromuscular pain, headache, dental pain, arthritis pain and pain caused by cancer.

As a result of their p38 inhibitory activity, compounds of the invention have utility in the prevention and treatment of diseases associated with cytokine production including but not limited to those diseases associated with TNF, IL-1, IL-6 and IL-8 production.

Thus TNF mediated diseases or conditions include for example rheumatoid arthritis, rheumatoid spondylitis, osteoarthritis, gouty arthritis and other arthritic conditions, sepsis, septic shock syndrome, adult respiratory distress

syndrome, cerebral malaria, chronic pulmonary inflammatory disease, silicosis, pulmonary sarcoiosis, bone resportion disease, reperfusion injury, graft vs. host reaction, allograft rejections, fever and myalgias due to infection, cachexia secondary to infection, AIDS, ARC or malignancy, keloid formation, scar tissue formation, Crohn's disease, ulcerative colitis, pyresis, viral infections such as HIV, CMV, influenza and herpes; and vetinary viral infections, such as lentivirus infections, including but not limited to equine infectious anemia virus, caprine arthritis virus, visna virus or maedi virus; or retrovirus infections, including feline immunodeficiency virus, bovine immunodeficiency virus or canine immunodeficiency virus.

Compounds of the invention may also be used in the treatment of viral infections, where such viruses elicit TNF production *in vivo* or are sensitive to upregulation by TNF. Such viruses include those that produce TNF as a result of infection and those that are sensitive to inhibition, for instance as a result of decreased replication, directly or indirectly by the TNF inhibiting compounds of the invention. Such viruses include, but are not limited to, HIV-1, HIV-2 and HIV-3, Cytomegalovirus (CMV), Influenza, adenovirus and the Herpes group of viruses such as Herpes Zoster and Herpes Simplex.

IL-1 mediated diseases or conditions include for example rheumatoid arthritis, osteoarthritis, psoriatic arthritis, traumatic arthritis, rubella arthritis, inflammatory bowel disease, stroke, endotoxemia and/or toxic shock syndrome, inflammatory reaction induced by endotoxin, diabetes, pancreatic β-cell disease, Alzheimer's disease, tuberculosis, atherosclerosis, muscle degeneration and cachexia.

IL-8 mediated diseases and conditions include for example those characterized by massive neutrophil infiltration such as psoriasis, inflammatory bowel disease, asthma, cardiac, brain and renal reperfusion injury, adult respiratory distress syndrome, thrombosis and

glomerulonephritis. The increased IL-8 production associated with each of these diseases is responsible for the chemotaxis of neutrophils into inflammatory sites. This is due to the unique property of IL-8 (in comparison to TNF, IL-1 and IL-6) of promoting neutrophil chemotaxis and activation. Therefore, inhibition of IL-8 production would lead to a direct reduction in neutrophil infiltration.

It is also known that both IL-6 and IL-8 are produced during rhinovirus (HRV) infections and contribute to the pathogenesis of the common cold and exacerbation of asthma associated with HRV infection [Turner *et al*, Clin. Infec. Dis., 1997, 26, 840; Grunberg *et al*, Am. J. Crit. Care Med. 1997, 155, 1362; Zhu *et al*, J. Clin. Invest. 1996, 97, 421]. It has also been demonstrated *in vitro* that infection of pulmonary epithelial cells (which represent the primary site of infection by HRV) with HRV results in production of IL-6 and IL-8 [Sabauste *et al*, J. Clin. Invest. 1995, 96, 549]. Therefore, p38 inhibitors of the invention may be used for the treatment or prophylaxis of the common cold or respiratory viral infection caused by human rhinovirus infection (HRV), other enteroviruses, coronavirus, influenza virus, parainfluenza virus, respiratory syncytial virus or adenovirus infection.

For the prophylaxis or treatment of a p38 or pro-inflammatory cytokine mediated disease the compounds according to the invention may be administered to a human or mammal as pharmaceutical compositions, and according to a further aspect of the invention we provide a pharmaceutical composition which comprises a compound of formula (1) together with one or more pharmaceutically acceptable carriers, excipients or diluents.

Pharmaceutical compositions according to the invention may take a form suitable for oral, buccal, parenteral, nasal, topical, ophthalmic or rectal administration, or a form suitable for administration by inhalation or insufflation.

For oral administration, the pharmaceutical compositions may take the form of, for example, tablets, lozenges or capsules prepared by conventional means with pharmaceutically acceptable excipients such as binding agents (e.g. pregelatinised maize starch, polyvinylpyrrolidone or hydroxypropyl methylcellulose); fillers (e.g. lactose, microcrystalline cellulose or calcium hydrogen phosphate); lubricants (e.g. magnesium stearate, talc or silica); disintegrants (e.g. potato starch or sodium glycollate); or wetting agents (e.g. sodium lauryl sulphate). The tablets may be coated by methods well known in the art. Liquid preparations for oral administration may take the form of, for example, solutions, syrups or suspensions, or they may be presented as a dry product for constitution with water or other suitable vehicle before use. Such liquid preparations may be prepared by conventional means with pharmaceutically acceptable additives such as suspending agents, emulsifying agents, non-aqueous vehicles and preservatives. The preparations may also contain buffer salts, flavouring, colouring and sweetening agents as appropriate.

Preparations for oral administration may be suitably formulated to give controlled release of the active compound.

For buccal administration the compositions may take the form of tablets or lozenges formulated in conventional manner.

The compounds for formula (1) may be formulated for parenteral administration by injection e.g. by bolus injection or infusion. Formulations for injection may be presented in unit dosage form, e.g. in glass ampoule or multi dose containers, e.g. glass vials. The compositions for injection may take such forms as suspensions, solutions or emulsions in oily or aqueous vehicles, and may contain formulatory agents such as suspending, stabilising, preserving and/or dispersing agents. Alternatively, the active

ingredient may be in powder form for constitution with a suitable vehicle, e.g. sterile pyrogen-free water, before use.

In addition to the formulations described above, the compounds of formula (1) may also be formulated as a depot preparation. Such long acting formulations may be administered by implantation or by intramuscular injection.

For nasal administration or administration by inhalation, the compounds for use according to the present invention are conveniently delivered in the form of an aerosol spray presentation for pressurised packs or a nebuliser, with the use of suitable propellant, e.g. dichlorodifluoromethane, trichlorofluoromethane, dichlorotetrafluoroethane, carbon dioxide or other suitable gas or mixture of gases.

The compositions may, if desired, be presented in a pack or dispenser device which may contain one or more unit dosage forms containing the active ingredient. The pack or dispensing device may be accompanied by instructions for administration.

For topical administration the compounds for use according to the present invention may be conveniently formulated in a suitable ointment containing the active component suspended or dissolved in one or more pharmaceutically acceptable carriers. Particular carriers include, for example, mineral oil, liquid petroleum, propylene glycol, polyoxyethylene, polyoxypropylene, emulsifying wax and water. Alternatively the compounds for use according to the present invention may be formulated in a suitable lotion containing the active component suspended or dissolved in one or more pharmaceutically acceptable carriers. Particular carriers include, for example mineral oil, sorbitan monostearate, polysorbate 60, cetyl esters wax, cetearyl alcohol, benzyl alcohol, 2-octyldodecanol and water.

For ophthalmic administration the compounds for use according to the present invention may be conveniently formulated as microionized suspensions in isotonic, pH adjusted sterile saline, either with or without a preservative such as bactericidal or fungicidal agent, for example phenylmercuric nitrate, benzylalkonium chloride or chlorhexidine acetate. Alternatively for ophthalmic administration compounds may be formulated in an ointment such as petrolatum.

For rectal administration the compounds for use according to the present invention may be conveniently formulated as suppositories. These can be prepared by mixing the active component with a suitable non-irritating excipient which is solid at room temperature but liquid at rectal temperature and so will melt in the rectum to release the active component. Such materials include for example cocoa butter, beeswax and polyethylene glycols.

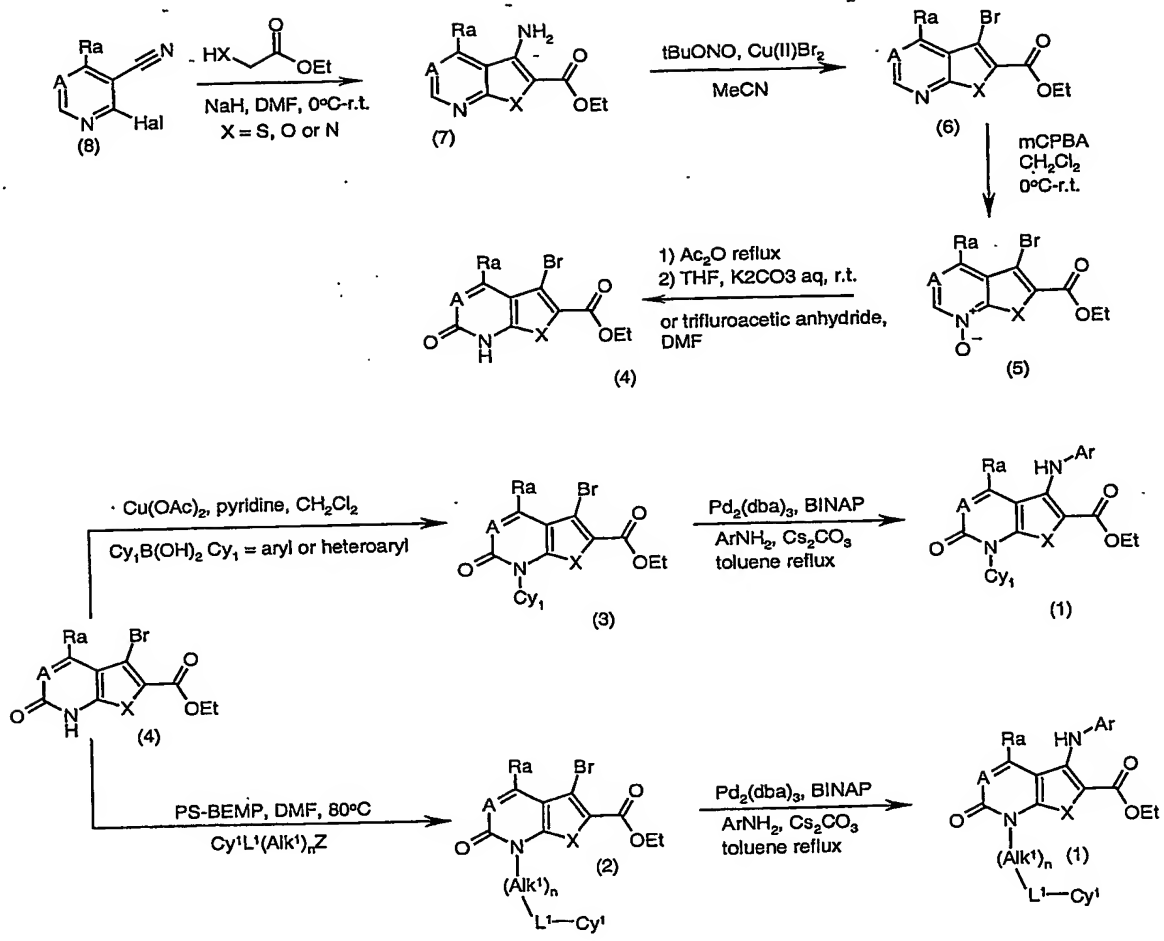
The quantity of a compound of the invention required for the prophylaxis or treatment of a particular condition will vary depending on the compound chosen, and the condition of the patient to be treated. In general, however, daily dosages may range from around 100ng/kg to 100mg/kg e.g. around 0.01mg/kg to 40mg/kg body weight for oral or buccal administration, from around 10ng/kg to 50mg/kg body weight for parenteral administration and around 0.05mg to around 1000mg e.g. around 0.5mg to around 1000mg for nasal administration or administration by inhalation or insufflation.

The compounds of the invention may be prepared by a number of processes as generally described below and more specifically in the Examples hereinafter. In the following process description, the symbols Ar, Cy^1, Alk^1, n, L^1, R, R^a, R^b, R^c, A, X and Y when used in the formulae depicted are to be understood to represent those groups described above in relation to formulae

(1a) and (1b) unless otherwise indicated. In the reactions described below, it may be necessary to protect reactive functional groups, for example hydroxy, amino, thio or carboxy groups, where these are desired in the final product, to avoid their unwanted participation in the reactions. Conventional protecting groups may be used in accordance with standard practice [see, for example, Green, T. W. in "Protective Groups in Organic Synthesis", John Wiley and Sons, 1999]. In some instances, deprotection may be the final step in the synthesis of a compound of formula (1) and the processes according to the invention described hereinafter are to be understood to extend to such removal of protecting groups.

Thus according to a further aspect of the invention a compound of formula (1) in which A is a $-C(R^b)=$ group, X is a –O- or –S- atom or –NH- group and Y is a substituted carbon atom in which the substituent is an esterified carboxyl group, for example a $-CO_2Alk^6$ group, may be prepared according to the reactions set out in Scheme 1 below. In the Scheme the preparation of an ethyl ester is specifically shown, but it will be appreciated that other esters may be obtained by simply varying the ester starting material and if appropriate any reaction conditions:

Scheme 1



Thus in Scheme 1 a compound of formula (1) may be prepared by reaction of a compound of formulae (2) or (3) with an amine $ArNH_2$ in the presence of a palladium catalyst. The reaction may be conveniently carried out in a solvent such as toluene at an elevated temperature, eg the reflux temperature, using a catalyst such as tris(dibenzylideneacetone)dipalladium(0), a phosphine ligand such as 2,2'-bis(diphenylphosphino)-1,1'-binaphthyl and a base such as caesium carbonate. Where desired, alternative reaction conditions may be used, for example as described in the literature [Luker et al. Tet. Lett. (2001) 41, 7731; Buchwald S.L. J.Org.Chem. (2000) 65 1144; Hartwig J.F. Angew. Chem. In. Ed. Engl. (1998) 37, 2046].

Intermediates of formula (2) may be prepared by reaction of a compound of formula (4) with an alkylating agent of formula $Cy^1L^1(Alk^1)_nZ$, where Z is a leaving group such as a halogen atom, e.g. a chlorine, bromine or iodine atom or a sulphonyloxy group such as an alkylsulphonyloxy e.g. trifluoromethylsulphonyloxy or arylsulphonyloxy e.g. phenylsulphonyloxy group.

The reaction may be performed in the presence of a solvent, for example a substituted amide such as dimethylformamide, optionally in the presence of a base, for example an inorganic base such as sodium hydride, or an organic base such as an organic amine, e.g. a cyclic amine such as 1,5-diazabicyclo[4.3.0]non-5-ene or a resin bound organic amine such as resin bound 2-*tert*-butylimino-2-diethylamino-1,3-dimethyl-perhydro-1,3,2-diazaphosphorine (PS-BEMP), at an elevated temperature, for example 80 to 100ºC.

Intermediates of formula (3) may be prepared by the reaction of a compound of formula (4) with a boronic acid of formula $Cy^1B(OH)_2$ in which Cy^1 is an aryl or heteroaryl group. The reaction may be performed in an organic solvent, for example a halogenated hydrocarbon such as dichloromethane or dichloroethane in the presence of a copper reagent, for example a copper (II) reagent such as copper (II) acetate, optionally in the presence of an oxidant, for example 2,2,6,6-tetramethyl-1-piperidinyloxy or pyridine-N-oxide, optionally in the presence of a base, for example an organic amine such as an alkylamine, e.g. triethylamine or an aromatic amine, e.g. pyridine at a temperature from around ambient to the reflux temperature [see for example Chan, D.T. *et al* Tetrahedron Letters, 1998, 2933; Lam, P.Y.S. *et al*, Tetrahedron Letters, 2001, 3415]

It will be appreciated that if desired the reactions just described may be carried out in the reverse order so that the amination using $ArNH_2$ is

performed first with the intermediate of formula (4) followed by alkylation/arylation to yield the compound of formula (1).

Intermediate pyridinones of formula (4) may be prepared from pyridine N-oxides of formula (5) by sequential reaction with an anhydride, for example acetic anhydride at an elevated temperature, for example the reflux temperature followed by reaction with an inorganic base, for example a carbonate such as aqueous potassium carbonate in a solvent such as an ether for example a cyclic ether e.g. tetrahydrofuran at around ambient temperature. Alternatively the reaction may be performed using trifluoroacetic anhydride in dimethylformamide from 0°C to ambient temperature conditions [see for example Konno et al., Heterocycles (1986) 24, 2169].

Pyridine N-oxides of formula (5) may be formed by oxidation of pyridines of formula (6) using an oxidising agent such as hydrogen peroxide in the presence of an acid such as acetic acid, at an elevated temperature, for example around 70ºC to 80ºC, or alternatively by reaction with a peracid such as peracetic acid or m-chloroperoxybenzoic acid in a solvent, such as a halogenated hydrocarbon e.g. dichloromethane or an alcohol e.g. tert-butanol at a temperature from the ambient temperature to the reflux temperature.

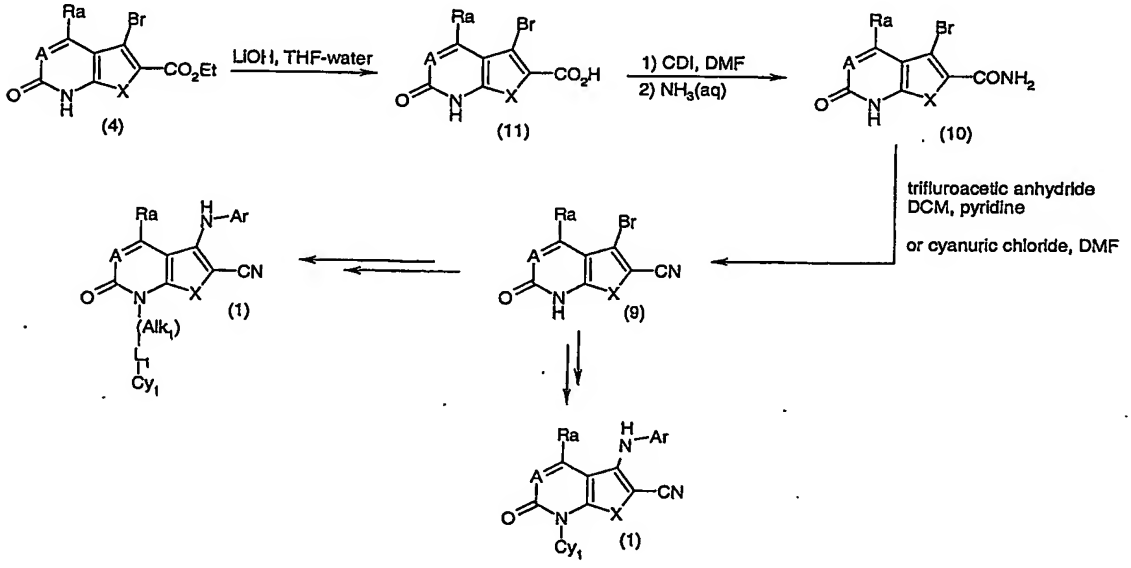
Intermediate pyridines of formula (6) in Scheme 1 may be obtained by standard methods such as for example by the Sandmeyer reaction. Thus for example a bromide of formula (6) may be prepared by treatment of an aryl amine of formula (7) with an alkyl nitrite, for example t-butyl nitrite and a copper salt, for example copper (II) bromide in the presence of a solvent, for example a nitrile such as acetonitrile at a temperature from about 0º to around 65ºC.

Amines of formula (7) may be formed from 2-halopyridine-2-carbonitriles of formula (8) by reaction with a reagent of formula $HXCH_2CO_2Et$ [where Et is

an ethyl group and X is a –O- or –S- atom or –NH- group]. The reaction may be performed in the presence of a solvent such as a substituted amide for example dimethylformamide or an ether e.g. a cyclic ether such as tetrahydrofuran or alcohol such as ethanol in the presence of a base, for example an inorganic base such as sodium carbonate or a hydride e.g. sodium hydride or an organic base such as 1,5-diazabicyclo[4.3.0]non-5-ene or a trialkylamine such as triethylamine at a temperature between about 0ºC and 100°C. The carbonitrile starting materials are readily available or may be obtained from known compounds using standard procedures.

In another process according to the invention, a compound of formula (1) in which A is a $-C(R^b)=$ group, X is a –O- or –S- atom or –NH- group and Y is a –C(CN)= group may be prepared using the reactions set out in Scheme 2 below:

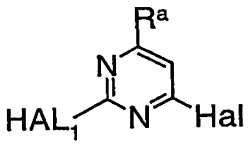
Scheme 2



Thus in Sceme 2, a 2-cyano intermediate of formula (9) may be aminated and alkylated or arylated in a final step to yield a compound of the invention

using the reactions and reagents described above in relation to the amination, alkylation and arylation of intermediates of formula (4). Nitriles of formula (9) may be obtained by dehydration of the corresponding amide of formula (10) using a dehydrating agent such as trifluoroacetic anhydride in the presence of a base such as pyridine in a solvent, for example a halogenated hydrocarbon such as dichloromethane at around ambient temperature. Alternatively, cyanuric chloride may be used in a solvent such as dimethylformamide at a temperature from around 0°C to 110°C. Amides of formula (10) may be obtained from the corresponding acids of formula (11) using conventional procedures, for example by reaction with 1,1'-carbonyldiimidazole and aqueous ammonia in a solvent such as dimethyl formamide at ambient temperature. The intermediate acids of formula (11) may be prepared by hydrolysis of esters of formula (4) using a base such as lithium hydroxide in water and a solvent such as tetrahydrofuran.

Compounds of formula (1) in which A is a –N= atom may be obtained using the synthetic routes in Schemes (1) and (2) with a pyrimidine starting material of formula (12):



(12)

wherein Hal_1 and Hal which may be the same or different is each a halogen atom such as a chlorine atom.

In this instance the final step in the synthesis of a compound of the invention may be hydrolysis of the Hal_1 atom using a base such as sodium or potassium hydroxide in a solvent such as an alcohol, e.g. methanol or

ethanol at an elevated temperature, e.g. the reflux temperature. Alternatively, the Hal_1 atom may first be converted to an ether by reaction with an alkoxide such as sodium methoxide or sodium benzyloxide in a solvent, e.g. an alcohol such as methanol or ethanol at a temperature between 0°C and the reflux, and the ether then cleaved using standard procedures such as by reduction with hydrogen gas in the presence of a catalyst such as a palladium catalyst, e.g. palladium on charcoal, or where the ether is an alkyl ether, by reaction with a trialkylsilyl halide such as trimethylsilyl chloride, optionally in the presence of an inorganic halide such as sodium iodide in a solvent such as a halogenated hydrocarbon, e.g. dichloromethane or in a nitrile e.g. acetonitrile.

Compounds of the invention and intermediates thereto where A represents a $-N(R^b)-$ or $-C(R^b)(R^c)-$ group may be generated from corresponding compounds of the invention or intermediates thereto where A represents a $-N=$ or $-C(R^b)=$ group by reduction, for instance by catalytic hydrogenation using a metal catalyst such as palladium on charcoal in the presence of hydrogen gas at an elevated pressure in a solvent such as an alcohol, e.g. ethanol optionally at an elevated temperaure e.g. between 40°C and 60ºC.

Where in the general processes described above intermediates such as alkylating agents of formula $Cy^1L^1(Alk^1)_nZ$, reagents of formula $HXCH_2CO2Et$ and any other intermediates required in the synthesis of compounds of the invention are not available commercially or known in the literature, they may be readily obtained from simpler known compounds by one or more standard synthetic methods employing substitution, oxidation, reduction or cleavage reactions. Particular substitution approaches include conventional alkylation, arylation, heteroarylation, acylation, thioacylation, halogenation, sulphonylation, nitration, formylation and coupling procedures. It will be appreciated that these methods may also be used to obtain or modify other intermediates and in particular compounds of formula (1) where appropriate

functional groups exist in these compounds. Particular examples of such methods are given in the Examples hereinafter.

Thus for example aromatic halogen substituents in the compounds may be subjected to halogen-metal exchange with a base, for example a lithium base such as n-butyl or t-butyl lithium, optionally at a low temperature, e.g. around -78°C, in a solvent such as tetrahydrofuran and then quenched with an electrophile to introduce a desired substituent. Thus, for example, a formyl group may be introduced by using dimethylformamide as the electrophile, a thiomethyl group may be introduced by using dimethyldisulphide as the electrophile, an alcohol group may be introduced by using an aldehyde as electrophile and an acid may be introduced by using carbon dioxide as electrophile. Aromatic acids of formula $ArCO_2H$ may also be generated by quenching Grignard reagents of formula ArMgHal with carbon dioxide.

Aromatic acids of formula $ArCO_2H$ generated by this method and acid containing compounds in general may be converted to activated derivatives, e.g. acid halides by reaction with a halogenating agent such as a thionyl halide e.g. thionyl chloride, a phosphorous trihalide such as phosphorous trichloride or a phosphorous pentahalide such as phosphorous pentachloride optionally in an inert solvent such as an aromatic hydrocarbon e.g. toluene or a chlorinated hydrocarbon e.g. dichloromethane at a temperature from about 0°C to the reflux temperature, or may be converted into Weinreb amides of formula ArC(O)N(OMe)Me by conversion to the acid halide as just described and subsequent reaction with an amine of formula HN(OMe)Me or a salt thereof, optionally in the presence of a base such as an organic amine, e.g. triethylamine in an inert solvent such as an aromatic hydrocarbon e.g. toluene or a chlorinated hydrocarbon e.g. dichloromethane at a temperature from about 0°C to ambient temperature.

Compounds of the invention and intermediates thereto may be prepared by alkylation, arylation or heteroarylation. For example, compounds containing a $-L^1H$ group (where L^1 is a linker atom or group) may be treated with an alkylating agent Cy^1Z^2 in which Z^2 is a leaving atom or group such as a halogen atom, e.g. a fluorine, chlorine, bromine or iodine atom or a sulphonyloxy group such as an alkylsulphonyloxy, e.g. trifluoromethylsulphonyloxy or arylsulphonyloxy, e.g. p-toluenesulphonyloxy group.

The reaction may be carried out in the presence of a base such as a carbonate, e.g. caesium or potassium carbonate, an alkoxide, e.g. potassium t-butoxide, or a hydride, e.g. sodium hydride, in a dipolar aprotic solvent such as an amide, e.g. a substituted amide such as dimethylformamide or an ether, e.g. a cyclic ether such as tetrahydrofuran.

In another example, compounds containing a $-L^2H$ group as defined above may be functionalised by acylation or thioacylation, for example by reaction with the alkylating agents just described but in which Z^2 is replaced by a $-C(O)Z^3$, $C(S)Z^3$, $-N(R^2)COZ^3$ or $-N(R^2)C(S)Z^3$ group in which Z^3 is a leaving atom or group as described for Z^2. The reaction may be performed in the presence of a base, such as a hydride, e.g. sodium hydride or an amine, e.g. triethylamine or N-methylmorpholine, in a solvent such as a halogenated hydrocarbon, e.g. dichloromethane or carbon tetrachloride or an amide, e.g. dimethylformamide, at for example ambient temperature. Alternatively, the acylation may be carried out under the same conditions with an acid (for example one of the alkylating agents described above in which Z^2 is replaced by a $-CO_2H$ group) in the presence of a condensing agent, for example a diimide such as 1-(3-dimethylaminopropyl)-3-ethylcarbodiimide or N,N'-dicyclohexylcarbodiimide, or a benzotriazole such as [O-(7-azabenzo-triazol-1-yl)-1,1,3,3-tetramethyluronium]hexafluorophosphate advantageously in the presence of a catalyst such as a N-hydroxy compound e.g. a N-

hydroxytriazole such as 1-hydroxybenzotriazole. Alternatively the acid may be reacted with a chloroformate, for example ethylchloroformate, prior to the desired acylation reaction

In a further example compounds may be obtained by sulphonylation of a compound containing an -OH group by reaction with one of the above alkylating agents but in which Z^2 is replaced by a -S(O)Hal or -SO_2Hal group [in which Hal is a halogen atom such as chlorine atom] in the presence of a base, for example an inorganic base such as sodium hydride in a solvent such as an amide, e.g. a substituted amide such as dimethylformamide at for example ambient temperature.

In another example, compounds containing a –L^2H group as defined above may be coupled with one of the alkylation agents just described but in which Z^2 is replaced by an -OH group in a solvent such as tetrahydrofuran in the presence of a phosphine, e.g. triphenylphosphine and an activator such as diethyl, diisopropyl- or dimethylazodicarboxylate.

Ester groups such as –CO_2Alk^6 and –CO_2R^4 in the compound of formula (1) and intermediates thereto may be converted to the corresponding acid [-CO_2H] by acid- or base-catalysed hydrolysis depending on the nature of the group Alk^6 or R^4. Acid- or base-catalysed hydrolysis may be achieved for example by treatment with an organic or inorganic acid, e.g. trifluoroacetic acid in an organic solvent e.g. dichloromethane or a mineral acid such as hydrochloric acid in a solvent such as dioxan or an alkali metal hydroxide, e.g. lithium hydroxide in an aqueous alcohol, e.g. aqueous methanol.

In a further example, -OR^6 [where R^6 represents an alkyl group such as methyl group] in compounds of formula (1) and intermediates thereto may be cleaved to the corresponding alcohol -OH by reaction with boron tribromide in

a solvent such as a halogenated hydrocarbon, e.g. dichloromethane at a low temperature, e.g. around -78°C.

Alcohol [-OH] groups may also be obtained by hydrogenation of a corresponding $-OCH_2R^{31}$ group (where R^{31} is an aryl group) using a metal catalyst, for example palladium on a support such as carbon in a solvent such as ethanol in the presence of ammonium formate, cyclohexadiene or hydrogen, from around ambient to the reflux temperature. In another example, -OH groups may be generated from the corresponding ester [e.g. $-CO_2Alk^6$] or aldehyde [-CHO] by reduction, using for example a complex metal hydride such as lithium aluminium hydride or sodium borohydride in a solvent such as methanol.

In another example, alcohol -OH groups in the compounds may be converted to a corresponding $-OR^6$ group by coupling with a reagent R^6OH in a solvent such as tetrahydrofuran in the presence of a phosphine, e.g. triphenylphosphine and an activator such as diethyl-, diisopropyl-, or dimethylazodicarboxylate.

Aminosulphonylamino [$-NHSO_2NH_2$] groups in the compounds may be obtained, in another example, by reaction of a corresponding amine [$-NH_2$] with sulphamide in the presence of an organic base such as pyridine at an elevated temperature, e.g. the reflux temperature.

In another example compounds containing a $-NHCSR^7$ or $-CSNHR^7$ group may be prepared by treating a corresponding compound containing a $-NHCOR^7$ or $-CONHR^7$ group with a thiation reagent, such as Lawesson's Reagent or P_2S_5, in an anhydrous solvent, for example a cyclic ether such as tetrahydrofuran, at an elevated temperature such as the reflux temperature.

In a further example amine ($-NH_2$) groups may be alkylated using a reductive alkylation process employing an aldehyde and a reducing agent. Suitable reducing agents include borohydrides for example sodium triacetoxyborohyride or sodium cyanoborohydride. The reduction may be carried out in a solvent such as a halogenated hydrocarbon, e.g. dichloromethane, a ketone such as acetone, or an alcohol, e.g. ethanol, where necessary in the presence of an acid such as acetic acid at around ambient temperature. Alternatively, the amine and aldehyde may be initially reacted in a solvent such as an aromatic hydrocarbon e.g. toluene and then subjected to hydrogenation in the presence of a metal catalyst, for example palladium on a support such as carbon, in a solvent such as an alcohol, e.g. ethanol.

In a further example, amine [$-NH_2$] groups in compounds of formula (1) and intermediates thereto may be obtained by hydrolysis from a corresponding imide by reaction with hydrazine in a solvent such as an alcohol, e.g. ethanol at ambient temperature.

In another example, a nitro [$-NO_2$] group may be reduced to an amine [$-NH_2$], for example by catalytic hydrogenation using for example hydrogen in the presence of a metal catalyst, for example palladium on a support such as carbon in a solvent such as an ether, e.g. tetrahydrofuran or an alcohol e.g. methanol, or by chemical reduction using for example a metal, e.g. tin or iron, in the presence of an acid such as hydrochloric acid.

In a further example amine ($-CH_2NH_2$) groups in compounds of formula (1) and intermediates thereto may be obtained by reduction of nitriles (-CN), for example by catalytic hydrogenation using for example hydrogen in the presence of a metal catalyst, for example palladium on a support such as carbon, or Raney® nickel, in a solvent such as an ether e.g. a cyclic ether such as tetrahydrofuran or an alcohol e.g. methanol or ethanol, optionally in

the presence of ammonia solution at a temperature from ambient to the reflux temperature, or by chemical reduction using for example a metal hydride e.g. lithium aluminium hydride, in a solvent such as an ether e.g. a cyclic ether such as tetrahydrofuran, at a temperature from 0°C to the reflux temperature.

In another example, sulphur atoms in the compounds, for example when present in a group L^1 or L^2 may be oxidised to the corresponding sulphoxide or sulphone using an oxidising agent such as a peroxy acid, e.g. 3-chloroperoxybenzoic acid, in an inert solvent such as a halogenated hydrocarbon, e.g. dichloromethane, at around ambient temperature.

In a further example N-oxides of compounds of formula (1) may in general be prepared for example by oxidation of the corresponding nitrogen base as described above in relation to the preparation of intermediates of formula (5).

Salts of compounds of formula (1) may be prepared by reaction of compounds of formula (1) with an appropriate base in a suitable solvent or mixture of solvents e.g. an organic solvent such as an ether e.g. diethylether, or an alcohol, e.g. ethanol using conventional procedures.

Where it is desired to obtain a particular enantiomer of a compound of formula (1) this may be produced from a corresponding mixture of enantiomers using any suitable conventional procedure for resolving enantiomers.

Thus for example diastereomeric derivatives, e.g. salts, may be produced by reaction of a mixture of enantiomers of formula (1) e.g. a racemate, and an appropriate chiral compound, e.g. a chiral base. The diastereomers may then be separated by any convenient means, for example by crystallisation and

the desired enantiomer recovered, e.g. by treatment with an acid in the instance where the diastereomer is a salt.

In another resolution process a racemate of formula (1) may be separated using chiral High Performance Liquid Chromatography. Alternatively, if desired a particular enantiomer may be obtained by using an appropriate chiral intermediate in one of the processes described above. Alternatively, a particular enantiomer may be obtained by performing an enantiomer specific enzymatic biotransformation e.g. an ester hydrolysis using an esterase and then purifying only the enantiomerically pure hydrolysed acid from the unreacted ester antipode.

Chromatography, recrystallisation and other conventional separation procedures may also be used with intermediates or final products where it is desired to obtain a particular geometric isomer of the invention.

The following Examples illustrate the invention. All temperatures are in °C. The following abbreviations are used:

NMM - N-methylmorpholine;
MeOH - methanol;
DCM - dichloromethane;
DIPEA - diisopropylethylamine;
Pyr - pyridine;
DMSO - dimethylsulphoxide;
Et_2O - diethylether;
THF - tetrahydrofuran;
MCPBA - 3-chloroperoxybenzoic acid;
FMOC - 9-fluorenylmethoxycarbonyl;
DBU - 1,8-Diazabicyclo[5,4-0]undec-7-ene;
EDC - 1-[3-(dimethylamino)propyl]-3-ethylcarbodiimide hydrochloride;
HOBT - 1-hydroxybenzotriazole hydrate;
EtOAc - ethyl acetate;
BOC - butoxycarbonyl;
AcOH - acetic acid;
EtOH - ethanol;
Ar - aryl;
iPr - isopropyl;
Me - methyl;
h – hour;
NBS - N-bromosuccinimide;
r.t. – room temperature;

BINAP - 2,2'-bis(diphenylphosphino)-1-1'-binaphthyl;
DMF - N,N-dimethylformamide;

All NMRs were obtained either at 300MHz or 400MHz.

Compounds were named with the aid of either Beilstein Autonom supplied by MDL Information Systems GmbH, Theodor-Heuss-Allee 108, D-60486 Frankfurt, Germany or ACD Labs Name (v.5.0) supplied by Avanced Chemical Development, Toronto, Canada.

LCMS retention times (RT) quoted were generated on a Hewlett Packard 1100 LC/MS using the following following method: Phenomenex Luna $3\mu C_{18}(2)$ 50x4.6mm column; mobile phase A = 0.1% formic acid in water; mobile phase B = 0.1% formic acid in MeCN; flow rate of $0.9mLmin^{-1}$, column temperature 40°C.

Gradient:-	Time	%B
	Initial	5
	2.00	95
	3.00	95
	5.0	5
	5.5	end

Intermediate 1

Ethyl 3-aminothieno[2,3-*b*]pyridine-2-carboxylate

A mixture of 2-chloro-3-cyanopyridine (330g), ethyl 2-mercaptoacetate (361.2g), sodium carbonate (265g) and EtOH (1.2L) was heated to reflux for 4.5 hours. It was then cooled to ambient temperature, added to water (10L) and the addition was washed in with water (5L). The resulting slurry was stirred for 30 minutes and then it was filtered. The filter cake was washed

with two portions of water (2 x 2.5L) and dried at the pump. The solids were then dried to constant weight under vacuum at 45°C to yield the title compound as a brown solid (493.1g, 93.2%). δH ($CDCl_3$) 8.68 (1H, dd, J 4.7, 1.2Hz), 7.93 (1H, dd, J 8.5, 1.2Hz), 7.29 (1H, dd, J 8.5, 4.7Hz), 5.90 (2H, b), 4.38 (2H, q, J 7.0Hz), 1.40 (3H, t, J 7.0Hz). LCMS RT 2.9 minutes, 223 $(M+H)^+$

Intermediate 2

Ethyl 3-bromothieno[2,3-*b*]pyridine-2-carboxylate

Intermediate 1 (363.6g) was added in portions over two hours to a mixture of copper(II) bromide (403.3g), t-butyl nitrite (220.6g) and acetonitrile (3.6L) stirred at a temperature of 20 to 25°C. The mixture was stirred at 20°C for 2 hours before it was slowly added to 2M HCl(aq) (4.2L). The reaction mixture slurry was filtered and the solids were washed with water (500mL). The combined filtrate was extracted with ethyl acetate (8L), this ethyl acetate solution was washed with 2M HCl(aq) (2.2L). The solids were dissolved in ethyl acetate (6L), this solution was washed twice with 2M HCl(aq) (4.4L and 2.2L). The two ethyl acetate solutions were then combined and washed with 2M HCl(aq) (2.2L) and twice with water (2 x 2L). The ethyl acetate solution was then dried ($MgSO_4$), filtered and concentrated *in vacuo* at 40 mbar and 60°C to give a solid residue. This was broken up and dried to constant weight under vacuum at 45°C to yield the title compound as a brown solid (458.5g, 97.9%). δH (DMSO-d6) 8.89 (1H, d, J 4.7Hz), 8.47 (1H, d, J 8.6Hz), 7.71 (1H, dd, J 8.6, 4.7Hz), 4.46 (2H, q, J 7.2Hz), 1.40 (3H, t, J 7.2Hz). LCMS RT 3.8 minutes, 288 $(M+H)^+$

Intermediate 3

Ethyl 3-Bromothieno[2,3-*b*]pyridine-2-carboxylate N-oxide

To a slurry of Intermediate 2 (214g, 0.747Mol) in DCM (2140mL) under nitrogen was added MCPBA (240g @ 70% = 168g, 0.97Mol) portion wise over 0.5h. The reaction was then stirred at room temperature for 18h. The

reaction mixture was quenched with water (800mL) and pH adjusted to 8.5 with 10%w/v sodium carbonate solution (1250mL). The basic aqueous layer was removed and the organic layer washed with water until pH 7. The organic layer was concentrated *in vacuo* and the crude title product was recovered as a tan solid. The crude product was purified by slurrying in MTBE (600mL) for 1h at 0-5°C to give the title compound (174g, 77%). δH ($CDCl_3$) 8.44 (1H, dd, J 6.2, 0.8Hz), 7.87 (1H, dd, J 8.3, 0.8Hz), 7.48 (1H, dd, J 8.3, 6.2Hz), 4.49 (2H, q, J 7.1Hz), 1.48 (3H, t, J 7.1Hz). LCMS (ES^+) RT 2.61 minutes, 302$(M+H)^+$

Intermediate 4

Ethyl 3-bromo-6-oxo-6,7-dihydrothieno[2,3-*b*]pyridine-2-carboxylate

A mixture of Intermediate 3 (500mg, 1.66mmol) and DMF (10mL) was set to stir at 0°C under nitrogen. To this reaction mixture was added trifluoroacetic anhydride (3.49g, 2.36mL, 16.6mmol) in one portion via syringe. After stirring for 16 hours the volatiles were removed *in vacuo* and the residue co-evaporated with toluene (2x20mL). The crude material was then extracted with EtOAc (2x100mL). The EtOAc extracts were dried ($MgSO_4$) and concentrated *in vacuo*. The crude product was purified by a re-slurry in toluene (10mL) to give the title compound as a beige solid (260mg, 52%). δH (DMSO-d6) 12.20 (1H, brs), 7.75 (1H, d, J 9.0Hz), 6.50 (1H, d, J 9.0Hz), 4.15 (2H, q, J 7.1Hz), 1.12 (3H, t, J 7.1Hz). LCMS (ES^+) RT 2.86 minutes, 302 ($(M+H)^+$, 100%). MP = 261.7-268.1°C.

Intermediate 5

Ethyl 3-bromo-6-oxo-7-phenyl-6,7-dihydrothieno[2,3-*b*]pyridine-2-carboxylate

To a 2 necked round bottomed flask was added in sequence Intermediate 4 (302mg, 1.00mmol), copper(II) acetate (278mg, 1.50mmol), phenylboronic acid (488mg, 4.00mmol), DCM (5mL) and pyridine (158mg, 2.00mmol). The reaction was stirred at room temperature for 18h with the exclusion of

moisture. The reaction was then diluted with DCM (50mL), washed with 2M HCl(aq) (50mL), the aqueous was re-extracted with DCM (50mL). The combined organics were then washed with water (50mL), dried ($MgSO_4$) and concentrated *in vacuo*. The crude product was purified by a slurry in methanol (12mL), to give the title compound as a beige solid (270mg, 72%). δH ($CDCl_3$) 7.82 (1H, d, J 8.5Hz), 7.70-7.62 (3H, m), 7.54-7.42 (2H, m), 6.70 (1H, d, J 8.5Hz), 4.15 (2H, q, J 7.1Hz), 1.14 (3H, t, J 7.1Hz). LCMS (ES^+) RT 3.75 minutes, 378 $(M+H)^+$. MP = 201.6-206.0°C.

Intermediate 6

3-Bromo-6-oxo-6,7-dihydrothieno[2,3-*b*]pyridine-2-carboxylic acid

Lithium hydroxide monohydrate (1.39g, 33.1mmol) was added to a suspension of Intermediate 4 (5.0g, 16.55mmol) in water (100mL) and the reaction stirred for 5 minutes. THF (10mL) was added and the reaction was stirred at r.t. for 18h. 2M HCl(aq) (40mL) was added to pH 1-2 and the resultant precipitate was collected by filtration, washed sparingly with EtOH and dried *in vacuo* to give the title compound as an off-white solid (4.5g). δH (DMSO-d6) 7.90 (1H, d, J 9.2Hz), 6.67 (1H, d, J 9.2Hz), pyridone and carboxylic acid protons not observed.

Intermediate 7

3-Bromo-6-oxo-6,7-dihydrothieno[2,3-*b*]pyridine-2-carboxamide

1,1'Carbonyldiimidazole (3.18g, 19.6mmol) was added to a suspension of intermediate 6 (4.30g, 15.7mmol) in anhydrous DMF (50mL) and the reaction stirred at r.t. under nitrogen until solution was achieved (30minutes). Ammonium hydroxide (50mL of 28% NH_3 in water) was added and the reaction stirred for 15 minutes before removing solvents *in vacuo*. The residue was suspended in water (75mL) and treated with 2M HCl(aq) (20mL). The resultant solid was collected by filtration, washed with water and dried in a vacuum oven to give the title compound as a pale brown solid (3.70g). δH

(DMSO-d6) 7.69 (1H, d, J 9.1Hz), 6.49 (1H, d, J 9.1Hz), 7.30 (1H, bs). LCMS (ES^+) 273 $(M+H)^+$.

Intermediate 8

3-Bromo-6-oxo-6,7-dihydrothieno[2,3-*b*]pyridine-2-carbonitrile

To a suspension of Intermediate 7 (3.70g, 13.55mmol) in DCM (200mL) was added pyridine (2.70mL, 34mmol) followed by trifluoroacetic anhydride (2.40mL, 17mmol). The reaction was stirred at r.t. for 8h before adding more trifluoroacetic anhydride (1.20mL, 8.5mmol). The reaction was stirred for a further 8h and was then concentrated *in vacuo*. The residue was suspended in water, acidified to pH 2 with 2M HCl(aq) and the resultant solid collected by filtration, washed with water and dried *in vacuo* to give the title compound as a pale yellow solid (3.20g). δH (DMSO-d6) 7.92 (1H, d, J 8.8Hz), 6.73 (1H, d, J 8.8Hz). LCMS (ES^+) 255 $(M+H)^+$.

Intermediate 9

3-bromo-6-oxo-7-phenyl-6,7-dihydrothieno[2,3-*b*]pyridine-2-carbonitrile

To an oven dried flask was added Intermediate 8 (2.0g, 7.84mmol), phenylboronic acid (1.19g, 15.7mmol), copper(II) acetate (1.42g, 7.84mmol), anhydrous pyridine (1.3mL, 16mmol) and anhydrous DCM (50mL). The reaction mixture was stirred at r.t. with the exclusion of moisture for 48h. The reaction was diluted with DCM (50mL), washed with 2M HCl(aq) (100mL), saturated $NaHCO_3$(aq) (50mL), dried (Na_2SO_4) and concentrated *in vacuo*. The residue was purified by chromatography on silica (5-10% EtOAc in DCM) to give the title compound as a white solid (1.24g). δH (DMSO-d6) 7.67 (1H, d, J 9.6Hz), 7.58-7.50 (3H, m), 7.32-7.29 (2H, m), 6.70 (1H, d, J 9.6Hz).

Example 1

Ethyl 3-(phenylamino)-6-oxo-7-phenyl-6,7-dihydrothieno[2,3-b]pyridine-2-carboxylate

Tris(dibenzylideneacetone)dipalladium(0) (12mg, 0.0133mmol, 5mol%) was added to a mixture of Intermediate 5 (100mg, 0.265mmol), caesium carbonate (120mg, 0.37mmol), aniline (0.030mL, 0.32mmol) and BINAP (17mg, 0.027mmol, 10mol%) in anhydrous toluene (2mL) and the reaction heated to reflux under nitrogen for 18h. Solvent was removed *in vacuo* and the crude residue purified by chromatography on silica (0-20% EtOAc in DCM) to give the title compound as a white solid (80mg). δH ($CDCl_3$) 8.70 (1H, bs), 7.57-7.47 (3H, m), 7.33-7.25 (4H, m), 7.20-7.10 (4H, m), 6.27 (1H, d, J 9.7Hz), 4.19 (2H, q, J 7.1Hz), 1.22 (3H, t, J 7.1Hz). LCMS (ES^+) RT 4.10 minutes, 391 $(M+H)^+$.

General procedure for the preparation of Ethyl 3-anilino-6-oxo-7-phenyl-6,7-tetrahydrothieno[2,3-*b*]pyridine-2-carboxylates

The compounds of Examples 2-13 were prepared by parallel synthesis using a Radleys Carousel reaction station (Radleys Ltd., Saffron Walden, U.K.) following a procedure similar to that described for Example 1. Therefore to each oven dried reaction tube in the Carousel was added a magnetic stirrer, the appropriate substituted aniline (0.64mmol), anhydrous toluene (3mL), Intermediate 5 (200mg, 0.53mmol), caesium carbonate (240mg, 0.74mmol) and tris(dibenzylideneacetone)dipalladium(0) (48mg, 0.053mmol, 10mol%). The reactions were heated to reflux under nitrogen and with magnetic stirring for 48h. Each reaction was then diluted with DCM (10mL), washed with water (10mL), dried ($MgSO_4$) and concentrated *in vacuo*. The crude products were purified on silica eluting with 0-20% EtOAc in DCM to give the title compounds as solids.

Example 2

Ethyl 3-[(2-chlorophenyl)amino]-6-oxo-7-phenyl-6,7-dihydrothieno[2,3-b]pyridine-2-carboxylate

From 2-chloroaniline to give the title compound (92mg). δH ($CDCl_3$) 8.60 (1H, bs), 7.56-7.48 (3H, m), 7.40-7.38 (1H, m), 7.36-7.32 (2H, m), 7.20-7.15 (2H, m), 7.14-7.05 (1H, m), 7.05-6.98(1H, m), 6.35 (1H, d, J 9.8Hz), 4.21 (2H q, J 7.1Hz), 1.23 (3H, t, J 7.1Hz). LCMS (ES^+) RT 4.38 minutes, 425 $(M+H)^+$.

Example 3

Ethyl 3-[(3-chlorophenyl)amino]-6-oxo-7-phenyl-6,7-dihydrothieno[2,3-b]pyridine-2-carboxylate

From 3-chloroaniline to give the title compound (65mg). δH ($CDCl_3$) 8.60 (1H, bs), 7.57-7.50 (3H, m), 7.36-7.30 (2H, m), 7.20-7.18 (1H, m), 7.18 (1H, d, J 9.7Hz), 7.05 (1H, d, J 1.5Hz), 7.05-7.04 (1H, m), 6.96-6.92 (1H, m), 6.35 (1H, d, J 9.8Hz), 4.19 (2H, q, J 7.1Hz), 1.22 (3H, t, J 7.1Hz). LCMS (ES^+) RT 4.30 minutes, 425 $(M+H)^+$.

Example 4

Ethyl 3-[(4-chlorophenyl)amino]-6-oxo-7-phenyl-6,7-dihydrothieno[2,3-b]pyridine-2-carboxylate

From 4-chloroaniline to give the title compound (115mg). δH ($CDCl_3$) 8.63 (1H, bs), 7.56-7.50 (3H, m), 7.34-7.31 (2H, m), 7.28-7.24 (2H, m), 7.12 (1H, d, J 9.8Hz), 7.02-6.99 (2H, m), 6.32 (1H, d, J 9.8Hz), 4.19 (2H, q, J 7.2Hz), 1.22 (3H, t, J 7.2Hz). LCMS (ES^+) RT 4.32 minutes, 425 $(M+H)^+$.

Example 5

Ethyl 3-[methyl(phenyl)amino]-6-oxo-7-phenyl-6,7-dihydrothieno[2,3-b]pyridine-2-carboxylate

From N-methylaniline to give the title compound (53mg). δH ($CDCl_3$) 7.61-7.43 (3H, m), 7.40-7.32 (2H, m), 7.26 (1H, d, J 9.6Hz), 7.22-7.10 (2H, m),

6.77 (1H, t, J 7.3Hz), 6.67 (2H, dd, J 8.7, 1.0Hz), 6.43 (1H, d, J 9.6Hz), 4.10 (2H, q, J 7.1Hz), 3.33 (3H, s), 1.11 (3H, t, J 7.1Hz). LCMS (ES^+) RT 4.01 minutes, 405 $(M+H)^+$.

Example 6

Ethyl 3-[(2-methoxyphenyl)amino]-6-oxo-7-phenyl-6,7-dihydrothieno[2,3-b]pyridine-2-carboxylate

From 2-methoxyaniline to give the title compound (133mg). δH ($CDCl_3$) 8.53 (1H, bs), 7.58-7.41 (3H, m), 7.36-7.29 (2H, m), 7.26 (1H, d, J 9.7Hz), 7.07-6.96 (2H, m), 6.89-6.75 (2H, m), 6.30 (1H, d, J 9.7Hz), 4.18 (2H, q, J 7.2Hz), 3.84 (3H, s), 1.22 (3H, t, J 7.2Hz). LCMS (ES^+) RT 4.06 minutes, 421 $(M+H)^+$.

Example 7

Ethyl 6-oxo-7-phenyl-3-[(3-trifluoromethoxyphenyl)amino]-6,7-dihydrothieno[2,3-b]pyridine-2-carboxylate

From 3-trifluoromethoxyaniline to give the title compound (60mg). δH ($CDCl_3$) 8.66 (1H, bs), 7.58-7.41 (3H, m), 7.34 (2H, d, J 8.0Hz), 7.28 (1H, t, J 8.3Hz), 7.20 (1H, d, J 9.8Hz), 6.98-6.93 (1H, m), 6.92-6.83 (2H, m), 6.35 (1H, d, J 9.8Hz), 4.19 (2H, q, J 7.1Hz), 1.22 (3H, t, J 7.1Hz). LCMS (ES^+) RT 4.39 minutes, 475 $(M+H)^+$.

Example 8

Ethyl 3-[(4-cyanophenyl)amino]-6-oxo-7-phenyl-6,7-dihydrothieno[2,3-b]pyridine-2-carboxylate

From 4-cyanoaniline to give the title compound (110mg). δH ($CDCl_3$) 8.59 (1H, bs), 7.61-7.45 (5H, m), 7.36-7.31 (2H, m), 7.28 (1H, d, J 9.7Hz), 7.02 (2H, d, J 8.6Hz), 6.43 (1H, d, J 9.7Hz), 4.20 (2H, q, J 7.1Hz), 1.23 (3H, t, J 7.1Hz). LCMS (ES^+) RT 3.71 minutes, 416 $(M+H)^+$.

Example 9

Ethyl 3-[(3-cyanophenyl)amino]-6-oxo-7-phenyl-6,7-dihydrothieno[2,3-b]pyridine-2-carboxylate

From 3-cyanoaniline to give the title compound (100mg). δH ($CDCl_3$) 8.58 (1H, bs), 7.61-7.43 (3H, m), 7.40-7.20 (6H, m), 7.14 (1H, d, J 9.8Hz), 6.38 (1H, d, J 9.8Hz), 4.19 (2H, q, J 7.1Hz), 1.23 (3H, t, J 7.1Hz). LCMS (ES^+) RT 3.78 minutes, 416 $(M+H)^+$.

Example 10

Ethyl 3-[(2-cyanophenyl)amino]-6-oxo-7-phenyl-6,7-dihydrothieno[2,3-b]pyridine-2-carboxylate

From 2-cyanoaniline to give the title compound (133mg). δH ($CDCl_3$) 8.72 (1H, bs), 7.61-7.47 (4H, m), 7.43-7.40 (1H, m), 7.36-7.31(2H, m), 7.22-7.15 (1H, m), 7.11-7.00 (2H, m), 6.40 (1H, d, J 9.8Hz), 4.22 (2H, q, J 7.1Hz), 1.24 (3H, t, J 7.1Hz). LCMS (ES^+) RT 3.80 minutes, 416 $(M+H)^+$.

Example 11

Ethyl 3-[(3-fluoro-4-methoxyphenyl)amino]-6-oxo-7-phenyl-6,7-dihydrothieno[2,3-b]pyridine-2-carboxylate

From 3-fluoro-4-methoxyaniline to give the title compound (122mg). δH ($CDCl_3$) 8.63 (1H, bs), 7.58-7.40 (3H, m), 7.32-7.25 (2H, m), 6.99 (1H, d, J 9.8Hz), 6.93-6.78 (3H, m), 6.28 (1H, d, J 9.8Hz), 4.18 (2H, q, J 7.1Hz), 3.85 (3H, s), 1.22 (3H, t, J 7.1Hz). LCMS (ES^+) RT 3.99 minutes, 439 $(M+H)^+$.

Example 12

Ethyl 3-[(2,4-difluorophenyl)amino]-6-oxo-7-phenyl-6,7-dihydrothieno[2,3-b]pyridine-2-carboxylate

From 2,4-difluoroaniline to give the title compound (99mg). δH ($CDCl_3$) 8.49 (1H, bs), 7.58-7.40 (3H, m), 7.32-7.25 (2H, m), 7.13-7.04(1H, m), 7.01 (1H, d,

J 9.8Hz), 6.93-6.86 (1H, m), 6.82-6.75 (1H, m), 6.31 (1H, d, J, 9.8Hz), 4.20 (2H, q, J 7.1Hz), 1.23 (3H, J 7.1Hz). LCMS (ES^+) RT 4.06 minutes, 427 $(M+H)^+$.

Example 13

Ethyl 6-oxo-7-phenyl-3-[(3-tolyl)amino]-6,7-dihydrothieno[2,3-b]pyridine-2-carboxylate

From 3-toluidine to give the title compound (95mg). δH ($CDCl_3$) 8.66 (1H, bs), 7.59-7.41 (3H, m), 7.36-7.27 (2H, m), 7.22-7.13 (1H, m), 7.11 (1H, d, J 9.8Hz), 6.95-6.84 (3H, m), 6.27 (1H, d, J 9.8Hz), 4.18 (2H, q, J 7.1Hz), 2.28 (3H, s), 1.22 (3H, t, J 7.1Hz). LCMS (ES^+) RT 4.36 minutes, 405 $(M+H)^+$.

Example 14

6-Oxo-3-(phenylamino)-7-phenyl-6,7-dihydrothieno[2,3-b]pyridine-2-carboxylic acid

Lithium hydroxide monohydrate (302mg, 7.2mmol) was added to a suspension of the compound of Example 1 (1.49g, 3.6mmol) in THF (20mL) and water (20mL) and the mixture heated at 60°C for 18h. The reaction was cooled to r.t. and bulk of THF removed *in vacuo*. The remaining concentrate was diluted with saturated ammonium chloride(aq) (50mL) and the solid precipitate filtered and washed with water (2x20mL), Et_2O (2x20mL) and dried *in vacuo* to give the title compound as a white solid in quantitative yield. LCMS (ES^+) RT 3.24 minutes, 363 $(M+H)^+$.

Example 15

3-(Phenylamino)-7-phenylthieno[2,3-b]pyridin-6(*7H*)-one

To a solution of the compound of Example 14 (200mg) in 1,4-dioxan (10mL) was added 2M HCl(aq) (0.5mL) and the reaction mixture heated at 70°C for 1h. The reaction was diluted with water (30mL), extracted with EtOAc (3x20mL) and the EtOAc extracts dried ($MgSO_4$) and concentrated *in vacuo*. The crude residue was purified by chromatography on silica (0-5% EtOAc in

DCM) to give the title compound as a white solid (90mg). δH (DMSO-d6) 8.21 (1H, bs), 7.96 (1H, d, J 9.6Hz), 7.63-7.47 (3H, m), 7.43-7.36 (2H, m), 7.25-7.11 (2H, m), 7.10-7.03 (2H, m), 6.82-6.71 (1H, m), 6.46 (1H, d, J 9.6Hz), 6.44 (1H, s). LCMS (ES^+) RT 3.54 minutes, 319 $(M+H)^+$.

Example 16

6-Oxo-3-(phenylamino)-7-phenyl-6,7-dihydrothieno[2,3-b]pyridine-2-carboxamide

To a suspension of the compound of Example 14 (370mg, 1.02mmol) in anhydrous DMF (5mL) was added 1,1'-carbonyldiimidazole (182mg, 1.12mmol) and the reaction stirred at r.t. under nitrogen for 20mins. Ammonium hydroxide (2mL of 28% NH_3 in water) was added and the reaction stirred for 72h. Solvents were removed *in vacuo* and the crude residue purified by chromatography on silica (0-15% THF in DCM) to give the title compound as a white solid (123mg). δH (DMSO-d6) 8.74 (1H, s), 7.67-7.34 (3H, m), 7.33-7.27 (2H, m), 7.22-7.00 (5H, m), 6.82-6.71 (3H, m), 6.21 (1H, d, J 9.7Hz). LCMS (ES^+) RT 3.04 minutes, 362 $(M+H)^+$.

Example 17

6-Oxo-*N*-(2-piperidinoethyl)-3-(phenylamino)-7-phenyl-6,7-dihydrothieno[2,3-b]pyridine-2-carboxamide

To a suspension of the compound of Example 14 (90mg, 0.23mmol) in DCM (2mL) was added EDC (60mg, 0.30mmol) and HOBT (41mg, 0.30mmol) and the mixture stirred at r.t. for 15 minutes. A solution 1-(2-aminoethyl)piperidine (45mg, 0.35mmol) in DCM (0.5mL) was added and the reaction stirred at r.t. for 18h. The reaction mixture was diluted with DCM (10mL), washed with water (2x5mL), dried ($MgSO_4$) and concentrated *in vacuo*. The crude product was purified by chromatography on silica (0-20%THF in DCM) to give the title compound as an off-white solid (23mg). δH ($CDCl_3$) 8.61 (1H, s), 7.61-7.43 (3H, m), 7.40-7.27 (2H, m), 7.25-7.13 (3H, m), 7.00-6.89 (3H, m), 6.50 (1H, bs), 6.33 (1H, d, J 9.7Hz), 3.43-3.25 (2H, m), 2.47-2.32 (2H, m), 2.31-2.11

(4H, m), 1.50-1.40 (4H, m), 1.39-1.25 (2H, m). LCMS (ES^+) RT 2.40 minutes, 473 $(M+H)^+$.

Example 18

6-Oxo-3-(phenylamino)-7-phenyl-6,7-dihydrothieno[2,3-b]pyridine-2-carbonitrile

Tris(dibenzylideneacetone)dipalladium(0) (34mg, 0.0375mmol, 5mol%) was added to a mixture of Intermediate 9 (250mg, 0.75mmol), caesium carbonate (342mg, 1.05mmol), aniline (0.082mL, 0.9mmol) and BINAP (47mg, 0.075mmol, 10mol%) in anhydrous toluene (7mL) and the reaction heated to reflux under nitrogen for 24h. The reaction mixture was partitioned between DCM (60mL) and water (25mL) and the DCM extracts dried (Na_2SO_4) and concentrated *in vacuo*. The crude residue was purified by chromatography on silica (10-15% EtOAc in DCM) to give the title compound as an off-white solid (185mg). δH ($CDCl_3$) 7.79-7.71 (3H, m), 7.56-7.41 (5H, m), 7.33-7.29 (1H, m), 7.24(2H, dd, J 7.5, 1.0Hz), 6.65 (1H, d, J 9.8Hz), 6.59 (1H, bs). LCMS (ES^+) 344 $(M+H)^+$.

Example 19

3-(3-Bromophenylamino)-6-oxo-7-phenyl-6,7-dihydrothieno[2,3-b]pyridine-2-carbonitrile

The title compound was prepared from 3-bromoaniline (155mg, 0.9mmol) following the method described for the compound of Example 18 to give the product as a pale yellow solid (183mg). δH ($CDCl_3$) 7.91-7.83 (3H, m), 7.83-7.66 (2H, m), 7.62 (1H, d, J 9.7Hz), 7.52-7.44 (3H, m), 7.23 (1H, dt, J 7.1, 1.7Hz), 6.81 (1H, d, J 9.7Hz), 6.74 (1H, bs). LCMS (ES^+) 422 $(M+H)^+$ ^{79}Br, 424 $(M+H)^+$ ^{81}Br.

Example 20

3-(3-chlorophenylamino)-6-oxo-7-phenyl-6,7-dihydrothieno[2,3-b]pyridine-2-carbonitrile

The title compound was prepared from 3-chloroaniline (115mg, 0.9mmol) following the method described for the compound of Example 18 to give the product as a pale yellow solid (125mg). δH ($CDCl_3$) 7.67-7.58 (3H, m), 7.44-7.41 (2H, m), 7.39 (1H, d, J 9.7Hz), 7.31-7.26 (1H, m), 7.12-7.09 (1H, m), 7.04 (1H, t, J 2.0Hz), 6.95-6.92 (1H, m), 6.57 (1H, d, J 9.7Hz), 6.54 (1H, bs). LCMS (ES^+) 378 $(M+H)^+$ ^{35}Cl, 380 $(M+H)^+$ ^{37}Cl.

The following assays and animal models can be used to demonstrate the potency and selectivity of the compounds according to the invention. In each assay an IC50 value was determined for each test compound and represents the concentration of compound necessary to achieve 50% inhibition.

Preparation of activated human p38α for inhibitor assays.

Purification of human p38α

Human p38α, incorporating an N-terminal (His)6 tag, was expressed in baculovirus-infected High-Five™ cells (Invitrogen) according to the manufacturers instructions. The cells were harvested 72h post-infection and lysed in phosphate buffered saline (PBS) containing 1% (w/v) β-octylglucoside and Complete, EDTA-free™ protease inhibitors (Roche Molecular Biochemicals). The lysate was centrifuged at 35000xg for 30min at 4oC and the supernatant applied to a NiNTA™ column (Qiagen). Bound protein was eluted by 150mM imidazole in PBS (after a wash with 15mM imidazole in PBS) and directly applied to a HiTrap Q™ column (AP Biotech). Bound protein was eluted using a 20 column volume, 0 to 1M NaCl gradient.

Fractions containing (His)6-p38 were aliquotted and stored at –70º prior to their activation.

Preparation of GST-MKK6EE-containing lysates

E. coli (BL21 pLysS) expressing the constituitively activated form of human MKK6 fused with an N-terminal glutathione–S-transferase tag (GST-MKK6EE) were harvested by centrifugation and frozen at –70º. Cells were lysed by resuspension in 1/10th the culture volume of PBS containing Complete, EDTA-free™ protease inhibitors followed by sonication on ice for 4x15 sec. Cell debris was removed by centrifugation at 35,000xg and the resultant supernatant stored in aliquots at –70º.

Activation of (His)6-p38

0.45mL of purified (His)6-p38 was incubated with 50µL of the GST-MKK6EE-containing lysate for 30min at 23º in the presence of 1mM β-glycerophosphate, 10mM $MgCl_2$ and 9mM ATP. The extent of activation was monitored by mass spectrometric detection of the doubly-phosphorylated form of (His)6-p38, which routinely comprised greater than 90% of the final (His)6-p38 preparation. The activated (His)6-p38 was then diluted x10 in PBS and repurified using the method described above. The concentration of purified, activated (His)6-p38 was measured by UV absorbance at 280nm using A280,0.1%=1.2 and the preparation stored in aliquots at –70º prior to its use in inhibitor assays.

p38 Inhibition Assays

Inhibition of phosphorylation of biotinylated myelin basic protein (MBP)

The inhibition of p38 catalysed phosphorylation of biotinylated MBP is measured using a DELFIA based format. The assay was performed in a buffer comprising, 20mM HEPES (pH 7.4), 5mM $MgCl_2$ and 3mM DTT. For a typical IC50 determination, biotinylated MBP (2.5µM) was incubated at room temperature in a streptavidin-coated microtitre plate together with activated

gst-p38 (10nM) and ATP (1μM) in the presence of a range of inhibitor concentrations (final concentration of DMSO is 2 percent). After fifteen minutes the reaction was terminated by the addition of EDTA (75mM). The microtitre plate was then washed with Tris buffered saline (TBS), prior to the addition of 100μl of anti-phospho MBP antibody (mouse) together with europium-labeled anti-mouse IgG antibody. After one hour at room temperature the plate was again washed in TBS followed by the addition of Enhancement solution (PerkinElmer Wallac). Fluorescence measurements were performed after a further fifteen minutes at room temperature.

IC50 values are determined from the plot of Log_{10} inhibitor concentration (x-axis) versus percentage inhibition of the fluorescence generated by a control sample in the absence of inhibitor (y-axis).

Purification of human Peripheral Blood Mononuclear Cells

Peripheral blood mononuclear cells (PBMC) were isolated from normal healthy volunteers. Whole blood was taken by venous puncture using heparinised vacutainers (Becton Dickinson), diluted 1 in 4 in RPMI 1640 (Gibco, UK) and centrifuged at 400g for 35 min over a Ficoll-paque gradient (Amersham-Pharmacia Biotech, UK). Cells at the interface were removed and washed once followed by a low speed spin (250g) to remove platelets. Cells were then resuspended in DMEM containing 10% FCS, penicillin 100 units ml^{-1}, streptomycin 50μg ml^{-1} and glutamine 2mM (Gibco, UK).

Inhibitor dilutions

Inhibitor stocks (20mM) were kept as a frozen solution (-20°C) in DMSO. Serial dilutions of inhibitors were performed in DMSO as 250-times concentrated stocks. Inhibitors were diluted 1 in 250 into tissue culture media, prewarmed to 37°C and transferred to plates containing PBMC. PBMC and inhibitors were incubated together for 30 mins prior to addition of LPS. Inhibitors used in whole blood assays were prepared according to a

different regime. Using the same stock solution serial dilutions of inhibitors were performed in DMSO. Inhibitors were then diluted 1 in 500 straight into whole blood in a volume of 1µL. Inhibitor was incubated with whole blood for 30 mins prior to the addition of LPS.

LPS stimulation of PBMC

PBMC were resuspended at a density of $2x10^5$ cells/well in flat bottomed 96 well tissue culture treated plates. After the addition of inhibitor cells were stimulated with an optimal dose of LPS (*E coli* strain B5:055, Sigma, at a final concentration of 1µg ml^{-1}) and incubated at 37°C in 5%CO_2/95% air for 18 hours. TNF-α levels were measured from cell free supernatants by sandwich ELISA (BioSource #CHC1751).

LPS stimulation of whole blood

Whole blood was taken by venous puncture using heparinised vacutainers (Becton Dickinson), and 500µl of blood aliquoted into each well of a 24 well tissue culture treated plate. After the addition of inhibitor cells were stimulated with an optimal dose of LPS (*E coli* strain B5:055, Sigma, at a final concentration of 1µg ml^{-1}) and incubated at 37°C without CO_2 for 18 hours. TNF-α levels were measured from cell free supernatants by sandwich ELISA (BioSource #CHC1751).

Rat LPS induced TNF release

Male Lewis rats (180-200g) are anaesthetised with Isofluor and injected i.v. with LPS* in a volume of 0.5ml sterile saline. After 90minutes blood is collected into EDTA tubes for preparation of plasma samples. Plasma is stored at –70°C prior to assay for TNFα by commercial ELISA.

Rat CIA

Female Lewis rats (180-200g) are anaesthetised with Isofluor and immunised i.d. at the base of the tail with 2x100µl of emulsion containing 4mg/ml bovine collagen II in 0.01M acetic acid and Freund's Incomplete Adjuvant at a ratio of 1:1. A polyarthritis develops with onset from about 13 days post sensitisation. The disease is mainly confined to the ankles and is quantified by plethysmometry. Results are expressed as change in paw volume over time.

In the p38 inhibitor assays described above compounds of the invention have IC_{50} values of around 1µM and below. The compounds of the invention are clearly potent inhibitors of p38 kinase, especially p38α kinase.